CHARACTERIZATION OF TAN SPOT FUNGUS POPULATIONS ON WHEAT IN OKLAHOMA

By

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Abstract: Wheat is the third most important crop in terms of planted land, production and value in the US. In Oklahoma, changes in cultural practices such as reduced tillage or no-till has contributed to a higher incidence of fungal leaf spot diseases. Tan spot caused by *Pyrenophora tritici-repentis* (PTR) is a significant wheat leaf spot disease capable of reducing yields in no-till systems. To evaluate the significance of PTR in Oklahoma, a culture-based, molecular identification approach was used to isolate and identify fungi from symptomatic leaves collected from 13 no-till winter wheat fields across Oklahoma during spring in 2016 and 2017. Cultures were morphologically identified as 'PTR-like' or 'pycnidial fungi'. Of 755 cultures isolated, 565 (75%) were PTR-like and 190 (25%) were pycnidial fungi. Molecular identification of 477 single-spore PTR-like isolates using PTR-specific PCR primers indicated that 311 (65%) of these were PTR in 2016, and from 139 PTR-like isolates 78 (56%) were PTR in 2017. Next, seven simple sequence repeat (SSR) markers were used to assess the genetic diversity of two subsets of PTR populations in Oklahoma. First, a subset of 180 PTR isolates from 10 fields in 2016 were found to show low genetic differentiation among field populations. And second, PTR isolates collected from four fields in 2016 and 2017 were found to show no genetic grouping of individuals according to geographic origin or year of sampling. Analysis of the genetic structure of PTR in 2016 and 2016-2017 suggests significant local distribution of the isolates, extensive long distance movement of the inoculum and frequent recombination. To evaluate the incidence of genes for the cultivar-specific toxins, ToxA and ToxB, among PTR isolates, specific primers were used. Of 389 isolates tested from 2016 and 2017, 373 (95%) were positive for *ToxA*, while none of the PTR isolates tested positive for *ToxB*. Phenotypic race characterization was performed using five PTR isolates on five differentials cultivars of wheat and found that four of the isolates were race 1 and the remaining one could not be classified as one of the eight known races. In summary, tan spot is the predominant leaf spot disease associated with no-till wheat in Oklahoma, populations of the tan spot fungus, PTR, undergo frequent genetic recombination and migration, and most PTR isolates appear to carry *ToxA* and can be classified as race 1.

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CHAPTER I

INTRODUCTION

Worldwide the three most important crops cultivated are maize, rice and wheat (FAO 2016). Wheat is one of the most important crops since it serves both human and animal consumption. This cereal is a fundamental source of carbohydrates, fiber and vitamins in the human diet (Shewry and Hey 2015). Because wheat can be cultivated over a wide range of climates and soils, it is grown in almost every region of the world (Atwell and Finnie 2016). The production of wheat worldwide in 2016 was 737 million metric tons, and in 2017 it was 753.1 million metric tons, which represents an increase of 2.18% from the previous year (USDA 2017).

In the United States, wheat is the third most important crop in terms of planted land, production and value, after corn and soybeans (Vocke and Ali 2013). The US total production of wheat in 2016 was 2.31 billion bushels, 12% more than in 2015 (NASS 2016). In the US, wheat is planted mainly in the North Central region, Southern Plains, Central Plains, Northern Plains and Pacific Northwest parts of the country (Vocke and Ali 2013). The main classes of wheat are: durum wheat, hard red spring wheat, hard red winter wheat, soft red winter wheat, hard white wheat, soft white wheat, unclassed wheat, and mixed wheat according to the U.S. standards. These classes are cultivated based on the soil and environmental conditions of each region (USDA 2014).

In Oklahoma, wheat constitutes a significant economic income with approximately 75% of the cropland in Oklahoma is dedicated to the cultivation of winter wheat (Patrignani et al. 2014). Hard red winter wheat is the predominant class planted in Oklahoma because it yields better than spring wheat in Oklahoma and because this type of wheat is compatible with Oklahoma's weather. Winter wheat is also planted in Oklahoma as a winter hay or pasture crop for cattle. Typically, winter wheat is planted during the fall (September to middle November) and is harvested during the spring (May to July) (USDA 2012).

However, as all other cereals, wheat is susceptible to a number of diseases that produce yield losses. Foliar diseases, often caused by fungi, represent one of the main concerns for producers (Williams and Littlefield 2004). In the Great Plains the most significant foliar diseases are leaf rust (*Puccinia triticina*), powdery mildew (*Blumeria graminis* f. sp. *graminis*), tan spot (*Pyrenophora tritici-repentis*), Septoria tritici blotch (*Zymoseptoria tritici* [syn. *Mycosphaerella graminicola*]), Spot blotch (*Bipolaris sorokiniana* [syn. *Cochliobolus sativus*]), and Stagonospora nodorum blotch (*Parastagonospora nodorum* [syn. *Phaeosphaeria nodorum*]). The severity of these diseases will depend on the environmental conditions, the cultural practices and the use of resistant cultivars (Wegulo et al. 2012).

Tan spot, caused by the pathogenic fungus *Pyrenophora tritici-repentis* (syn. *Drechslera tritici-repentis*), is a major leaf spot disease of wheat all around the world. In the southern region of United States, where winter wheat is largely cultivated, tan spot is an important disease that can produce significant yield and grain quality losses (Evans et al. 1999). Usually, 5% to 10% yield loss is caused by tan spot; however, this disease can cause from 20% to 50% yield losses when suitable environmental conditions are present (Sharma 2012; Wegulo 2011).

In Oklahoma, tan spot was reported in the late 1970s but was not considered a severe threat to the wheat productivity. However, the incidence of this disease has increased since the 1970s. Changes in cultivation practices are the main reason for the increase of residue-borne diseases, since growers have been adopting conservational tillage or no-till for wheat cultivation (Kader 2010; Sharma 2012). These practices have been accepted in attempts to reduce soil erosion, to increase organic matter content of soils, to reduce input costs, and to maximize soil water conservation. However, minimum tillage and no-till can increase the prevalence, incidence and severity of tan spot and other diseases because crop debris is left on the soil surface, increasing the inoculum available to initiate epidemics (Carignano et al. 2008; Wegulo et al. 2012). Furthermore, the conducive habitat of *P. tritici-repentis* overlaps with that of the pycnidial fungi *Parastagonospora nodorum* and *Zymoseptoria tritici*, forming a disease complex that can produce severe economic losses (McMullen and Adhikari 2009).

This project aimed to assess the incidence and relevance of fungi causing leaf spots of wheat in Oklahoma through isolation in culture and morphologically discriminating *Pyrenophora tritici-repentis*-like isolates from isolates of the pycnidial species *Parastagonospora nodorum* and *Zymoseptoria tritici*. Based on this initial survey of wheat leaf spot fungi, *P. tritici-repentis* (PTR) was determined to be the predominant pathogen causing leaf spots in Oklahoma. Hence, the second aim of this study was to analyze the population structure of PTR across the wheat-producing areas of Oklahoma. Finally, toxin production by PTR isolates was examined to identify the races present. This fundamental information should help the future development of resistant cultivars and best disease management practices.

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CHAPTER II

LITERATURE REVIEW

Tan spot of wheat

Wheat (*Triticum aestivum* L.) is a major staple food for humans and animals and thus, one of the most important cereal crops worldwide (Awika 2011). Wheat is consumed around the world by approximately two billion people since it is a good source of carbohydrates, vitamins and minerals (Kader 2010; Shewry and Hey 2015). Its nutritional value, and the fact that this crop can be cultivated in a variety of soils and environments, makes it a valuable crop around the world (Atwell and Finnie 2016).

Wheat is cultivated in the US in almost every state, but especially in the Great Plains (Wishart 2004). Oklahoma is one of the major winter wheat producing states in the US. Wheat is an economically important crop for Oklahoma, where it generated an economic revenue of $47 million in 2016 (NASS 2016). Due to historical problems of soil erosion during the dust bowl years of the 1930s, conservation tillage and no-till have progressively been adopted by growers in the state as practices to minimize soil erosion and to conserve water and soil (Schuh 1990). Changes in cultural practices, such as shorter crop rotations and the use of susceptible cultivars combined with reduced tillage or no-till, have resulted in the increase of the incidence of fungal diseases (Mergoum et al. 2007).

Foliar diseases are among the most significant diseases of wheat since they can cause significant yield losses (Williams and Littlefield 2004). Tan spot, also known as

yellow leaf spot, is caused by the ascomycete *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph: *Drechslera tritici-repentis (Died.) Shoemaker*) and is a major foliar disease of wheat around the world (Figure 2.1). Lately, tan spot has become a serious concern in Oklahoma (Ali et al. 2010). Tan spot is an economically important fungal disease that can cause up to 50% yield loss, when environmental conditions are suitable, thus causing devastating economic impacts to wheat producers (Wegulo et al. 2012a). In Oklahoma, tan spot yield losses of up to 28% have been documented in Stillwater when no fungicides were applied (Williams and Littlefield 2004). Tan spot decreases the photosynthetic area of leaves, which in turn affects the plant entirely. The lost photosynthesis and stress on wheat plants reduces the number of kernels per head, their size and weight. Furthermore, plants infected by this fungal pathogen are shorter, have less tillers and a reduced biomass (Faris et al. 2013; Sharma 2012).

Figure 2.1 Global distribution of tan spot (*Pyrenophora tritici-repentis*) of wheat. The disease has been reported in the countries highlighted in grey, and the numbers represent the different races identified (Lamari and Strelkov 2010).

Symptoms

Tan spot symptoms can occur on wheat during all growth stages. Symptoms will differ according to wheat genotype, pathogen race and weather conditions (Sharma 2012). Typical symptoms for tan spot are observed mainly on the leaves, but also are observed on infected kernels. Initial symptoms appear on the lower leaves, showing small oval, yellow-shaped lesions randomly on the foliar area. These symptoms are mostly observed during the late winter and spring. As the disease progresses, lesions enlarge and become tan and often are surrounded by a yellow halo (McMullen and Adhikari 2009). When disease is severe, lesions merge, leaves turn yellow, senesce and die (Wolf 2008b). On seeds, the fungus discolors the seed coat, and is known as red smudge. Infected seeds shrivel and have a low germination rates. Epidemiologically, seed infection is not relevant, but it can cause some market discounts due to dockage when selling discolored grain (McMullen and Adhikari 2009; Sharma 2012).

Pycnidial fungi

Tan spot has been often confused with other diseases because the symptoms caused by other foliar pathogens are very similar. Also, reports have shown that tan spot occurs often as a complex with Stagonospora nodorum blotch and Septoria tritici blotch. This leaf-spot complex is capable of reducing grain yield and quality (Duveiller et al. 1998; McMullen and Adhikari 2009).

Parastagonospora nodorum (Berk.) Quaedvlieg, Verkley & Crous is an important wheat pathogen responsible for causing the disease known as Stagonospora nodorum blotch. This fungal pathogen has a wide host range and can infect multiple species of grasses. *P. nodorum* is a leaf spot fungus that causes symptoms similar to those of tan spot

and Septoria tritici blotch, therefore the identification of this pathogen in the field can be difficult. The main symptoms are dark, brown, round lesions on the leaves with a surrounding yellow halo that converge as the disease develops. The formation of pycnidia (black fruiting bodies) in the mature lesions is one of the main differences from tan spot; however, these are very difficult to see for the untrained eye and are only present in later stages of the disease (Freije and Wise 2015; Solomon et al. 2006).

Zymoseptoria tritici (Desm.) Quaedvlieg & Crous is the causal agent of Septoria tritici blotch on wheat. The main symptoms of this disease are dark brown blotches on the leaves. One distinctive feature of this disease is the presence of pycnidia within the blotches. Early in the season Septoria tritici blotch and Stagonospora nodorum blotch are easily confused and laboratory microscopic examination is required to provide an accurate diagnosis (Hollaway 2014; Wolf 2008a).

Management

Management and control of tan spot can be challenging due to the pathogen's biology and ease of dispersal. However, an integrated disease management strategy that combines chemical, cultural and genetic approaches has proven to be effective to prevent the disease or to manage it to reduce losses. (Moreno et al. 2012).

Chemical control is widely used in wheat producing states to prevent yield losses and to generate higher economic revenues. Foliar fungicides can be used to shield the leaves from fungal spores and to prevent infection (McMullen and Adhikari 2009). Therefore, a combination of local and systemic fungicides is usually recommended to control the disease.

The two major classes of local and systemic fungicides used for foliar diseases are the strobilurins and triazoles, respectively. Strobilurins are local fungicides that are quinone outside inhibitors (QoI). These compounds impede spore germination, which is an effective mechanism to prevent infections. Examples of QoI fungicides registered for the use in wheat are azoxystrobin, pyraclostrobin and trifloxystrobin. On the other hand, the triazoles are curative fungicides that move through the xylem. They act by altering sterol biosynthesis, making it a reliable compound to treat early infections (Duveiller et al. 1998). Examples of triazoles used in the US to manage fungal diseases of wheat are metconazole, propiconazole, prothioconazole, and tebuconazole (Lopez et al. 2014; Wegulo et al. 2012b). If disease severity on lower leaves is low, fungicide typically should be applied during flag leaf and head emergence to obtain optimum protection of the plant during grain fill (Wolf 2008b).

Host plant resistance is another strategy commonly used to manage tan spot. The use of resistant cultivars is an affordable, effective and environmentally friendly management alternative for this disease. Several studies have reported different ploidy groups of the genus *Triticum* that seem to confer resistance to tan spot. Additionally, tan spot resistance has also been found in wild species related to wheat (Singh 2001; Tadesse et al. 2006). Currently, resistant durum and bread wheat cultivars are used in the US; however, some of the cultivars commonly planted are susceptible (Sun et al. 2010; Wegulo 2011).

Cultural methods can also be used to manage tan spot in order to reduce the amount of initial inoculum. Crop rotations with non-hosts, burning crop residue, removal of straw, or tillage are some of the most used approaches, since they eliminate the inoculum in debris left on the soil, preventing infections and consequently epidemics (Raymond et al. 1985;

Singh 2001). Additionally, since the disease can also be transmitted by infected seed, seed treatments are recommended (McMullen and Adhikari 2009).

Pyrenophora tritici-repentis (PTR)

Classification and Host Range

Pyrenophora tritici-repentis belongs to the Superkingdom: Eukaryota; Kingdom: Fungi; Phylum: Ascomycota; Subphylum: Pezizomycotina; Class: Dothidiomycetes; Subclass: Pleosporomycetidae; Order: Pleosporales; Family: Pleosporaceae; Genus: *Pyrenophora*; Species: *tritici-repentis* (Crous et al. 2004; Wegulo 2011).

Pyrenophora tritici-repentis has a wide host range of perennial grasses. It can infect at least 26 plant species where the pathogen can overwinter, acting as inoculum reservoirs, becoming a serious problem for cereal cultivation (Sharma 2012). Importantly, *P. tritici-repentis* infects economically significant grasses such as wheat, barley and rye, as well as several other species (Wegulo 2011).

Disease cycle

The disease cycle of this fungal pathogen starts in the infected stubble or residue left in the field from the previous year, where the pseudothecia (fruiting bodies) form. The pseudothecia releases ascospores (sexual spores), which are forcibly discharged and dispersed by wind. Ascospores serve as primary inoculum. Asexual spores, also known as conidia, are produced by the fungus on pseudothecia as well as, on lesions. Conidia usually serve as secondary inoculum, but, depending on the environmental conditions, can act as primary inoculum. Conidia are released into the air and will be dispersed by wind longer distances than the ascospores due to their light weight. Once spores reach the leaves, they

germinate, the pathogen starts to grow and infect the leaves, which is followed by appearance of symptoms. At the end of the cropping season, the pathogen will overwinter/oversummer in the stubble as pseudothecia that restart the disease cycle the next season (Figure 2.2) (Wegulo 2011; Wolf 2008b).

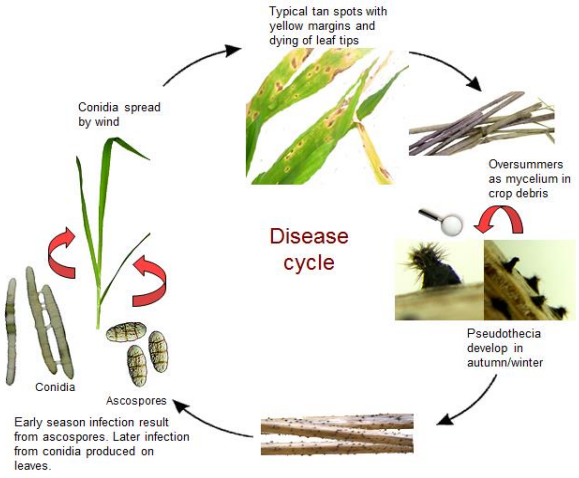

Figure 2.2. Tan spot disease cycle on wheat (Department of Primary Industries and Regional Development, 2016).

The infection process begins once the spores have reached the leaves surfaces and form germ tubes, which then form appressoria. Penetration pegs start to develop from the appressoria and penetrate intracellularly through stomata or between epidermal cells or intracellularly into epidermal cells by mechanical and enzymatic methods, forming an intracellular vesicle. From intercellular hyphae or intracellular vesicle, fungal growth continues into the mesophyll layer, destroying cell organelles by the production of toxins, and disrupting cell walls (Kim et al. 2013; Sharma 2012; Wegulo 2011).

Environmental conditions play an important role in the dispersal and development of the disease. Moisture and humidity can increase the incidence and prevalence of the

fungus. Temperatures from 10 °C to 30 °C, with 6 to 48 hours of free moisture, favor disease development (Moreno et al. 2012).

Pathogen Races

Efforts to classify *P. tritici-repentis* have improved through the years, resulting from a better understanding of the interaction between the pathogen and its host. Lamari and Bernier (1989) first suggested a classification considering the lesion types that were produced by the pathogen. Later, the same authors proposed a system based on four pathotypes to classify *P. tritici-repentis* isolates according to the symptoms produced on wheat cultivars (Lamari and Bernier 1989). However, when cultivars thought to be resistant to all isolates were found showing symptoms a race designation system was implemented (Andrie et al. 2007).

At present *P. tritici-repentis* races are classified according to the symptoms caused by the production of host selective toxins on differential wheat lines (Ali and Francl 2003). The characterization of these eight races is performed using five differential wheat cultivars, Glenlea, Katepwa and Salamouni, and the lines 6B365 and 6B662 (Andrie et al. 2007). Race 1 induces necrosis on Glenlea and Katepwa, and chlorosis on line 6B365; race 2 causes necrosis on Glenlea and Katepwa but does not produce chlorosis on line 6B365; race 3 causes chlorosis only on line 6B365; race 4 is considered avirulent, meaning that it does not produce either symptom on any of the differentials; race 5 induces chlorosis on Katepwa and line 6B662; race 6 combines the virulences of races 3 and 5, causing chlorosis on Katepwa and lines 6B662 and 6B365; race 7 combines the virulences of races 2 and 5, causing necrosis on Glenlea and Katepwa and chlorosis on Katepwa and line 6B662; race 8 combines the virulences of races 2, 3 and 5 causing necrosis on Glenlea and Katepwa and

chlorosis on Katepwa and lines 6B662 and 6B365 (Andrie et al. 2007; Effertz et al. 2002; Friesen et al. 2005).

In North America, races 1 and 2 are predominant, but all 5 have been reported in the United States. Race 5 is mainly found in North Africa and Algeria. Race 6 has been reported in Algeria (Strelkov et al. 2002) while races 7 and 8 have been identified from Azerbaidjan, Syria, Turkey and South America (Lamari et al. 2003; Moreno et al. 2012; Shaukat and Francl 2002).

Host-selective toxins

The pathogenicity of *Pyrenophora tritici-repentis* depends principally on host selective toxins (HSTs) produced by the fungus. The typical tan spot symptoms of necrosis and chlorosis, or the combination of both, are associated with these fungal toxins. Until recently, three toxins have been described, Ptr ToxA, Ptr ToxB and Ptr ToxC; however, it is believed that there are at least two more HST produced by the pathogen based on symptoms on wheat differentials (Strelkov et al. 2006).

Ptr ToxA was the first Ptr toxin described and is responsible for host plant necrosis produced by races 1, 2, 7 and 8 (Sharma 2012). ToxA is a 13.2 kDa protein encoded by a single copy gene. Cell death is caused by this toxin due to the accumulation of reactive oxygen species (ROS) in chloroplasts, which alters photosynthesis homeostasis (Ciuffetti et al. 2010; Manning et al. 2009; Moreno et al. 2012; Pandelova et al. 2012). Evidence has demonstrated that the gene responsible for the production of ToxA was acquired though horizontal gene transfer from *Stagonospora nodorum* allowing *P. tritici-repentis* to produce Ptr ToxA (Faris et al. 2013).

Ptr ToxB and Ptr ToxC are both chlorosis-inducing proteins but on different cultivars. ToxB is a 6.5kDa protein encoded by a multiple-copy gene. This toxin, which is produced by races 5, 6, 7 and 8, has not had its mode of action fully described. However, studies have shown that its pathogenicity is light dependent and that interferes with photosynthesis by accumulation of ROS possibly leading to chlorophyll photooxidation (Pandelova et al. 2012).

Ptr Tox C has been only partially characterized, but studies suggest it is a polar, low molecular weight, non-protein host selective toxin (Antoni et al. 2010; Effertz et al. 2002; Manning and Ciuffetti 2015). This toxin has been found in race 1 but data indicates the possibility that races 1, 3, 6 and 8 can also produce it (Friesen et al. 2005; Sharma 2012).

Genetic diversity

Genetic diversity studies of plant pathogen populations are essential to a better understanding of the disease epidemiology, to develop resistant cultivars, and to develop better management practices. As *P. tritici-repentis* is a homothallic fungus, it is able to reproduce sexually or asexually, which confers to this pathogen the ability to recombine, producing new genotypes that can be easily dispersed as airborne conidia (Kader 2010). Likewise, reports have shown that the pathogen's populations on wheat and alternative hosts are variable in terms of virulence and aggressiveness (Ali and Francl 2003).

Population studies have shown high levels of polymorphisms among *P. tritici-repentis* isolates but no correlation between random amplified polymorphic DNA (RAPD) polymorphism and geographic origin, production of toxins, or pathogenicity (Santos et al. 2002; Singh and Hughes 2006). Similarly, previous reports documented high levels of

variability with no genetic grouping of the races by geographic location, which suggested that the pathogen outcrosses in nature (Friesen et al. 2005).

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CHAPTER III

A CULTURE-BASED SURVEY OF THE WHEAT LEAF SPOT FUNGI IN OKLAHOMA AND MOLECULAR IDENTIFICATION OF *Pyrenophora tritici-repentis* CULTURES

INTRODUCTION

Tan spot caused by *Pyrenophora tritici-repentis* (PTR), Stagonospora nodorum blotch (SNB) caused by *Parastagonospora nodorum* and Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* are the three most commonly occurring leaf spotting diseases of wheat in North America (Singh et al. 2006). In the past few years, the incidence of leaf spotting diseases has increased in the central part of the United States, where the main wheat growing states are found, including Texas, Colorado, Oklahoma, Kansas, Nebraska, South Dakota, and North Dakota (Carignano et al. 2008).

The disease complex formed by tan spot, STB and SNB represent a serious concern for wheat production, since this complex can cause up to 20 to 50% yield loss when environmental conditions are suitable, especially when the flag leaf is damaged (Salgado and Paul 2016; Singh and Hughes 2006). The disease complex affects principally the photosynthetic area of the leaves, that results in a reduction in the number of tillers and kernels, shriveling of the kernels, and poor grain quality (Osman et al. 2016). Furthermore, the associated fungal pathogens produce similar symptoms on the leaf surface, leading to

misidentification. They are also able to infect the same host simultaneously, causing devastating effects for wheat producers (Freije and Wise 2015; Osman et al. 2016) .

Changes in cultural practices, such as reduced tillage or no-till, planting susceptible varieties, not rotating out of wheat or using a short crop rotation are the main reasons behind the increase of these fungal diseases, because inoculum persists in the residue until the next growing season (Moreno et al. 2008; Singh et al. 2006). Cropping with conservational tillage has increased in Oklahoma with aims to reduce soil erosion and maximize soil moisture, which increases the likelihood of severe epidemics of leaf spotting diseases (Edwards et al. 2006).

Efforts to control leaf-spotting diseases requires accurate diagnosis, which can be challenging because these fungal pathogens produce similar lesions; therefore, field identification of the specific causal agent by visual observation is complicated (Beck and Ligon 1995; Engle et al. 2006; Singh et al. 2006). This complicates selection of management strategies, such as selection of resistant cultivars because cultivars vary in their susceptibility to the different fungi, or the most efficient chemical treatment as some pathogens can be less sensitive to certain fungicides (Beck and Ligon 1995). Thus, knowing the incidence of these pathogens in wheat fields is fundamental to developing resistant varieties and improving practices to manage fungal diseases.

The first goal of this study was to enumerate morphologically identified PTR-like cultures and pycnidia-forming cultures *P.nodorum* and *Z. tritici* from symptomatic wheat samples from 13 no-till wheat fields, to determine the incidence of these pathogens in Oklahoma. The second goal of this study was to confirm the identity of single-spore isolates from PTR-like cultures as PTR using species-specific PCR.

MATERIALS AND METHODS

Sampling

Leaf samples showing tan spot symptoms were randomly collected from 13 no-till wheat fields in the main wheat producing areas in Oklahoma in spring (March to May) of 2016 (Figure 3.1). In 2017, four of these 13 no-till wheat fields were selected for a second year of sampling (Figure 3.2). In each field five different sampling spots were selected. From each sampling spot, five leaves were collected from different wheat plants. In total 325 leaves were collected in 2016 and 100 leaves were collected in 2017. The leaves were stored in ziplock bags and transported in a cooler back to the laboratory at OSU, Stillwater. Table 3.1 and Table 3.2 details collection dates, locations (field, neighboring town, and county), and wheat varieties sampled in 2016 and 2017 respectively.

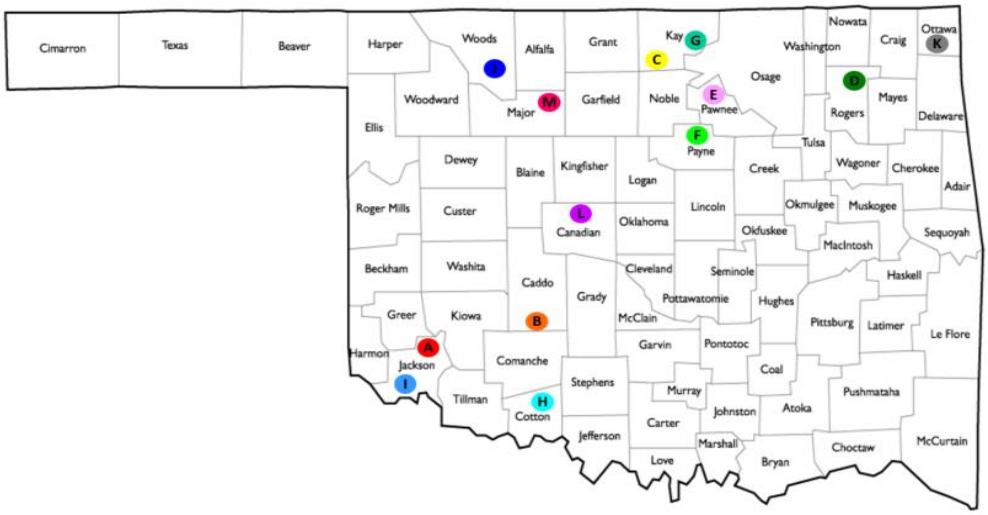


Figure 3.1. Oklahoma map depicting the locations of 13 no-till wheat fields sampled during 2016.

Table 3.1. Oklahoma fields sampled in 2016

COLLECTION DATE	FIELD	LOCATION	COUNTY	VARIETY
03/17/2016	A	Altus	Jackson	Gallagher
03/17/2016	B	Apache	Caddo	Gallagher
03/30/2016	C	Scheiber	Kay	Gallagher, Billings, Endurance
04/08/2016	D	Talala	Rogers	Everest, Cedar
04/08/2016	E	Pawnee	Pawnee	Unknown
04/08/2016	F	Stillwater	Payne	Unknown
04/25/2016	G	Kildare	Kay	Unknown
04/26/2016	H	Walters	Cotton	Duster
04/26/2016	I	Olustee	Jackson	Fuller
05/04/2016	J	Alva	Woods	Duster
05/06/2016	K	Afton	Ottawa	Ruby Lee, Iba
05/10/2016	L	Banner Road	Canadian	Gallagher
05/16/2016	M	Lahoma	Major	Oakley, Billings, Smith's Gold

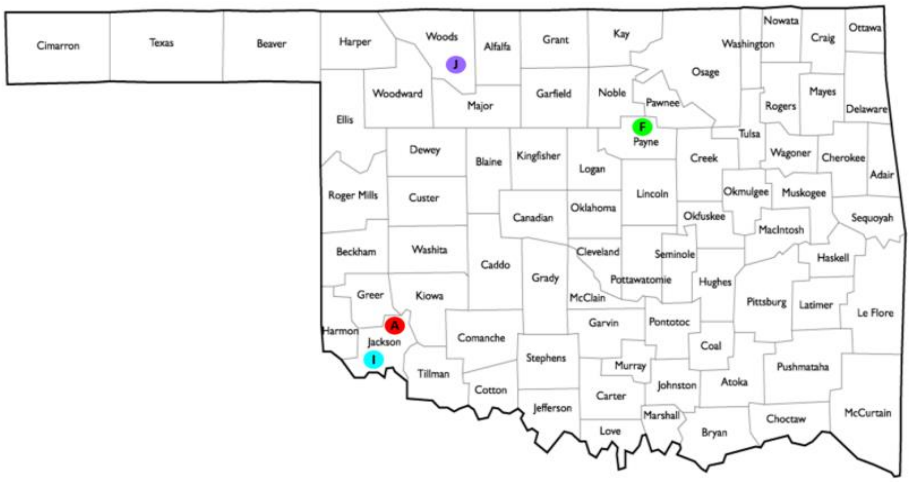


Figure 3.2. Oklahoma map depicting the locations of four no-till wheat fields sampled during 2017.

Table 3.2. Oklahoma fields sampled in 2017

COLLECTION DATE	FIELD	LOCATION	COUNTY	VARIETY
04/13/2017	A	Altus	Jackson	Gallagher
04/18/2017	F	Stillwater	Payne	Unknown
05/19/2017	I	Olustee	Jackson	Fuller
05/17/2017	J	Alva	Woods	Duster

Isolation of fungal pathogens

Wheat leaves were surface sterilized by immersion in 90% ethanol for 30s, followed by 10% bleach solution for 30s, cut into 1 cm pieces, and three to six pieces placed on yeast peptone sucrose (YPS) agar with antibiotics (0.1% yeast, 0.1% tryptone, 0.1% sucrose, 0.01% chloramphenicol, 0.02% penicillin G, 1.7% agar) in a 9 cm diameter plates. Plated samples were incubated under constant light conditions at room temperature for 7 to 10 days. Fungal morphology was examined to discriminate between PTR-like isolates and pycnidial species and enumerated. PTR-like cultures and pycnidial cultures each were hyphal-tip-isolated onto YPS agar in a 9 cm plate with a 7 cm filter paper on it. After incubating room temperature until mycelia covered 80-100% of the filter papers (7 to 14 d), the filter papers removed, dehydrated in a clean 9 cm plate in a desiccator for two weeks, and then stored in 2-0z.Whirl Pack (Nasco, Fort Atkinson, WI) bags at 4°C with silica gel desiccant.

From the initial collection of PTR-like isolates, single spore isolation was performed. For each PTR-like isolate, a piece (~1 cm^2) of dessicated filter paper culture was placed on V8 juice agar (1.63% V8-juice, 0.163% calcium carbonate, 1.8% agar) and incubated at room temperature. After ten days of growth, 1000 µl of sterile water was added to each culture plate and the mycelia matted down using a spreader. Then cultures plates were incubated at room temperature under constant light for 24 h to induce conidiophore formation. Next, these plate were incubated at 16 °C in the dark for 24h for the production of conidia. Conidia were streaked on water agar (2.0% agar) and incubated at room temperature under light for 24h to induce spore germination. Three single germinated spore of each culture were picked up using a needle, transferred to 9 cm potato dextrose agar (PDA) plate with filter paper and incubated at room temperature for seven to

ten days for mycelia growth. The filter paper was dehydrated and stored as previously described.

DNA extraction

DNA extraction from PTR-like isolates was performed directly from filter paper using the PowerLyzer Ultra Clean Microbial DNA Isolation Kit (MO BIO, Carlsbad, CA) following the manufacturer instructions. CTAB Protocol I (Weising et al. 2005) was used when the isolated DNA's quality or concentration obtained with the commercial kit was poor. Slight modifications were made for the CTAB protocol. Briefly, a small piece (~2 cm^2) of dried filter paper culture with mycelium was transferred to a 2 ml microtubes containing one 6 mm glass bead, three 3 mm glass beads and 100 µl 0.5 mm zirconium beads. The tubes were inserted in liquid nitrogen for 5 sec and then homogenized by bead-beating for 20 sec at 4 m/s in a FastPrep® FP 120 instrument (MP Biomedicals, Santa Ana, CA) three times. In each tube, 1 ml of extraction buffer (140 mM sorbitol, 220 mM Tris-HCl pH 8.0, 22 mM EDTA, 800 mM NaCl, 0.8% CTAB and 1% sodium dodecyl sulfate) was added. After homogenization, 500 µl of chloroform: isoamyl alcohol (24:1, v/v) was added and incubated in a Thermomixer (Eppendorf AG, Hamburg, Germany) for 30 min at 58°C, with constant shaking at 500 RPM. Then tubes were centrifuged at 12,000 × *g* at 4°C for 15 min. The upper aqueous phase was transferred to new tubes and DNA was precipitated by adding 750 µl of isopropanol and incubating the tubes at -20° C for 30 min. After DNA precipitation, the tubes were centrifuged at 14,000 x g for 8 min and the supernatant was discarded. The pellet was air dried and resuspended in 50 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). DNA concentration and quality (A 260:280 and

260:230 ratios) were measured using a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Molecular identification

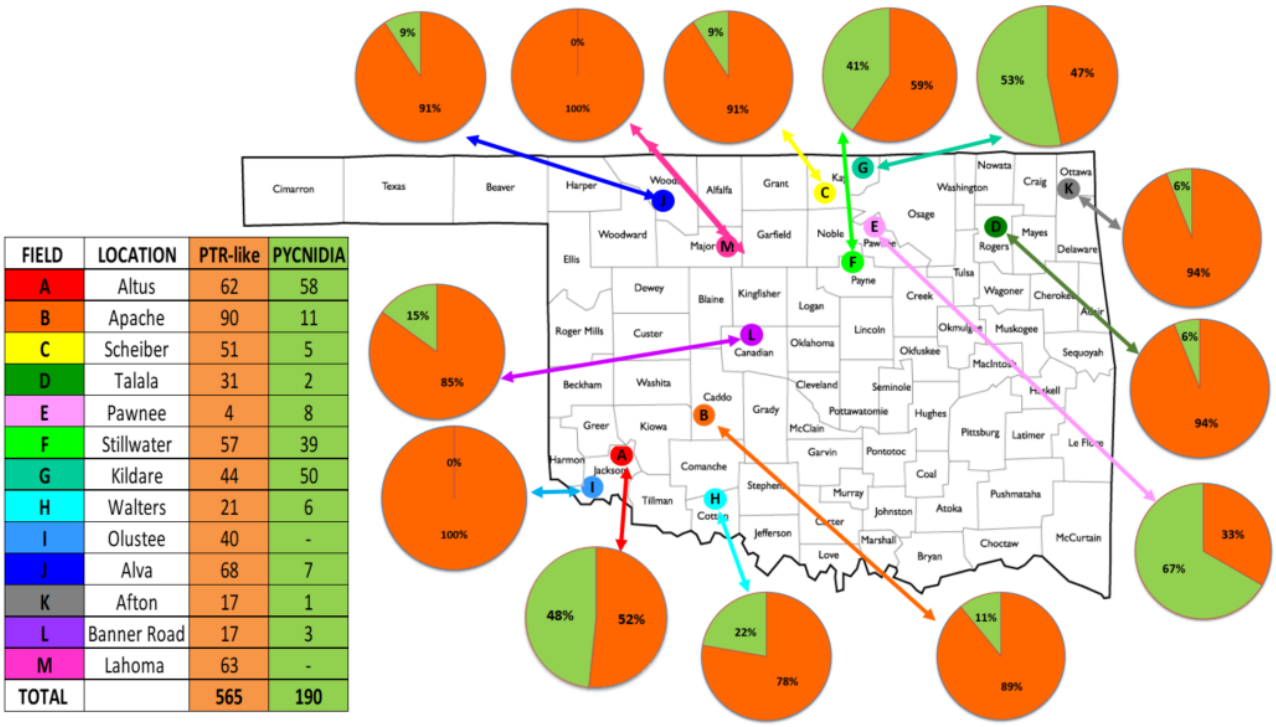
Previously reported species specific primers for the molecular identification of PTR were used to confirm the identity of PTR-like isolates (Antoni et al. 2010). PTRUniqueF2 (5´GGACTTTGGCTTTCTATTGTGC3´) and PtrUniqueR2 (5´CTTGGTGAATGGTGAAGATGG3´) produced a 490 bp amplicon. PCR amplification was performed in 20 µl reactions using 5 ng of template DNA, 10 µl of 2X GoTaq Green Master Mix (Promega, Madison, WI), 6 µl of nuclease-free water, and 2 µl of each 5 µM primer. The PCR program was 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 57°C for 30s, and 72°C for 1 min; with a final extension at 72°C for 10 min. PCR were performed in a PTC-200 thermal cycler (Marshall Scientific, Hampton, NH). Electrophoresis of PCR products was performed on EtBr stained 2% agarose gels, run at 100 V for 1 hour in TAE buffer. DNA fragments were observed in a GelDoc-It Imaging System and analyzed using VisionWorks LS software (UVP, LLC, Upland, CA).

RESULTS

Fungal Isolation

The incidence of tan spot versus STB and SNB in Oklahoma was determined based on symptoms and morphological characteristics of the fungi associated to leaf symptoms. In 2016 a total of 755 isolates were obtained from 13 no-till wheat fields sampled in the main wheat producing areas of the state. From these, the cultural morphology of 565 isolates corresponded to PTR-like organisms (74.83%) and 190 isolates (25.16%) corresponded to the pycnidial fungi, *P. nodorum* or Z. *tritici* (Figure 3.3). In each of the 13

fields evaluated, the number of recovered isolates of PTR-like and pycnidial species varied; but, overall, in most of the fields PTR was the prevalent species (Figure 3.3). Only three fields, in Altus, Stillwater and Kildare, showed a large number of pycnidial species in 2016. Based on these results, PTR or PTR-like species appear to cause most of the wheat leaf spot disease in no-till wheat fields in Oklahoma in 2016.



FIELD	LOCATION	PTR-like	PYCNIDIA
A	Altus	62	58
B	Apache	90	11
C	Scheiber	51	5
D	Talala	31	2
E	Pawnee	4	8
F	Stillwater	57	39
G	Kildare	44	50
H	Walters	21	6
I	Olustee	40	-
J	Alva	68	7
K	Afton	17	1
L	Banner Road	17	3
M	Lahoma	63	-
TOTAL		565	190

Figure 3.3. Morphological identification of isolates as PTR-like or pycnidial species obtained from 13 no-till wheat fields in Oklahoma in 2016, and the number of isolates of each type per field.

Molecular Identification

Of the 565 PTR-like isolates in 2016, tentatively identifies by cultural morphology, 477 PTR isolates were recovered using single-spore isolation. From these single spore isolates, 311 (65.19%) were confirmed as PTR by PCR with PTR species-specific primers (Figure 3.4). From the four fields re-sampled in 2017 (Altus, Stillwater, Olustee and Alva),

a total of 139 PTR-like single spore isolates were obtained, and 78 (56.11%) (see supplementary table 3) of these were identified as PTR (Figure 3.5). The identity of PTR-like isolates that were PCR-negative using species-specific primers was not examined further.

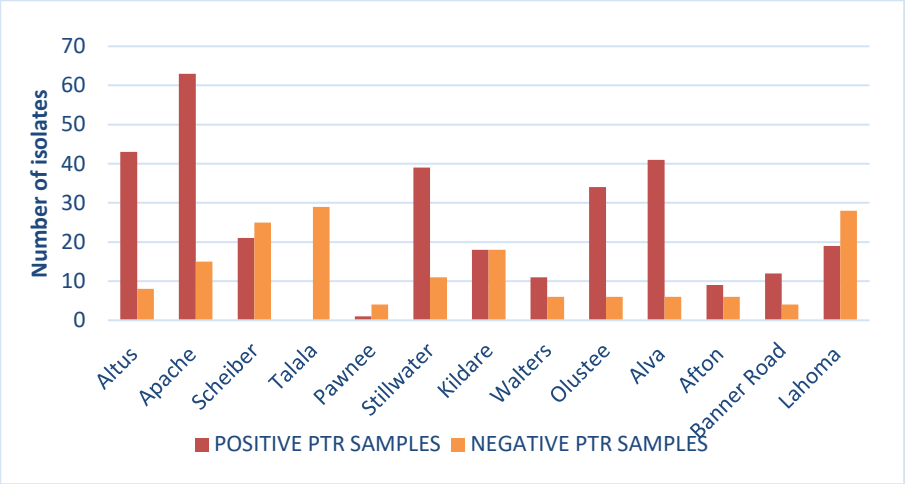


Figure 3.4. PCR-positive and –negative, single-spore PTR isolates isolated using species specific primers, obtained from 13 no-till wheat fields in Oklahoma in 2016.

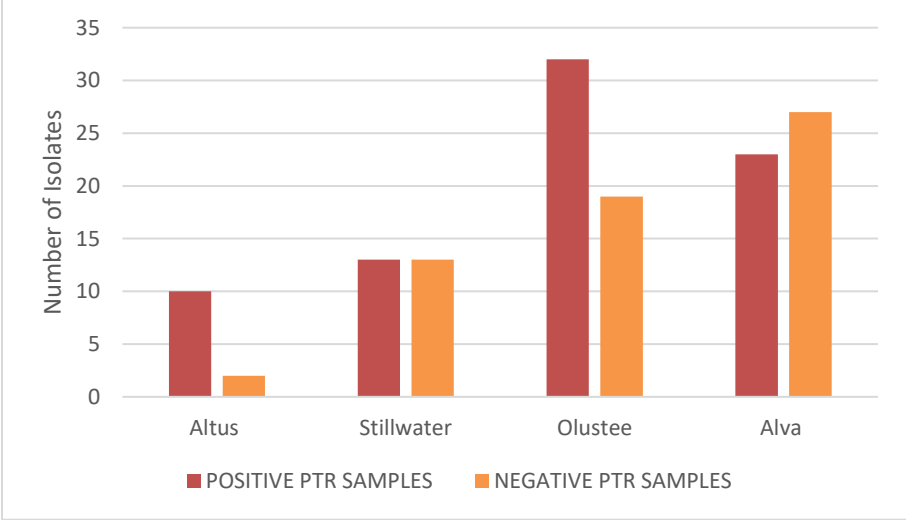


Figure 3.5. PCR-positive and -negative PTR isolates, identified using species-specific primers, obtained from four no-till wheat fields in Oklahoma in 2017.

DISCUSSION

Accurate species identification is essential to classify and determine the taxonomy of plant pathogens, as well as to study their epidemiology. Historically, morphological characteristics have been used to classify fungal species and different organisms (Guarro et al. 1999). In mycology, the Saccardo system has been used in the past for identification of several fungal species. Although this method is currently no longer used to infer systematic relationships among fungal taxa, it is still used routinely for identification of unknown fungi (Barnett and Hunter 1998). Morphological features used for identification of fungal species may vary from species to species; but, generally, fruiting structures, spore morphology, and pigmentation of conidia and conidiophores, are taken into consideration when identifying fungi (Barnett and Hunter 1998). For the identification of PTR-like isolates and pycnidial species, morphological features such as hyphal structure, the shape and coloration conidiomata, conidiophores and conidia were observed to conduct a preliminary identification of the fungal pathogens. From a collection of 755 isolates, 74.83% corresponded to PTR-like organisms and 25.16% to pycnidial species based only on their morphology. Based on these results, tan spot was the predominant leaf spot disease of wheat in Oklahoma, with the remaining leaf spots attributed to STB and SNB in 2016. This agrees with previous findings, where among all by fungal species contributing to the wheat leaf spot disease complex, tan spot often predominates when wheat residue is left on the soil surface (Wegulo 2011).

By contrast, other studies have determined that the occurrence and development of diseases caused by fungal pathogens often depend on environmental conditions found in a geographic area (Engle et al. 2006). Although, environmental conditions play an important role in plant disease epidemics for tan spot, STB, and SNB, we were not able to determine a

pattern that could explain the presence of higher numbers of PTR-like isolates, compared to pycnidial species. Consequently, further analysis should be conducted to determine the specific environmental causes favoring the higher amount of PTR-like isolates compared to the pycnidial species.

Although morphological characteristics are a useful method to differentiate fungal species, molecular identification using DNA sequences methods have proven to be a more reliable for identifying fungi, which often fail to produce key morphologic characters in culture (Raja et al. 2017). Single spore isolation was performed for PTR-like fungal isolates prior to molecular identification. From the 565 PTR-like isolates, only 477 single-spore cultures could be isolated. The lower number of single-spore isolates obtained was expected since isolates spores do not always germinate on artificial media (Choi et al. 1999), and persistent contamination with bacteria, yeasts or other filamentous fungi prevent the isolation of pure fungal cultures (Goh 1999).

Species-specific primers confirmed the identity of 311 (65.19%) single-spore isolates as PTR. It is possible that the isolates that did not amplify using PTR species-specific primers corresponded to other, related, genera. Species in the sexual genus *Pyrenophora* and asexual genus *Drechslera* were formerly included in *Helminthosporium* because of the similar conidial morphologies (Nelson 1994). Furthermore, this *Helminthosporium* complex also included later segregated genera such as *Cochliobolus, Bipolaris, Setosphaeria* and *Curvularia,* that also shared similar conidial characteristics with the genus *Pyrenophora* (Razzaghi-Abyaneh et al. 2015; Zhang and Berbee 2001). Currently, these genera have been revised to confirm with "one fungus, one name" nomenclature rules and are currently taxonomically segregated into the genera *Bipolaris* (*Cochliobolus*), *Exserohilum* (*Setosphaeria*), *Curvularia* (*Pseudocochliobolus*) and

Pyrenophora (*Drechslera*) (Manamgoda et al. 2014; Rossman et al. 2015). Even though these fungal pathogens are now classified in different genera, their often-formed conidial morphologies can appear very similar, which can lead to misidentification. *Pyrenophora* and *Bipolaris* have been often confused due to their very similar symptoms on wheat seedlings and leaves and their similar conidial morphology (Razzaghi-Abyaneh et al. 2015; Santos et al. 2002); therefore, it is possible that *Bipolaris* species could have been mixed in the leaf spot samples and PTR-like isolates. Furthermore, *Bipolaris sorokiniana*, causal agent of spot blotch, has also been found as part of the wheat leaf spot disease complex comprised of tan spot, STB and SNB, increasing the probability of encountering *Bipolaris* species among our isolates (Singh and Hughes 2003; Wegulo 2011). Further molecular identification would be required to determine accurately all the genera in these samples.

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CHAPTER IV

CHARACTERIZATION OF THE POPULATION STRUCTURE OF *Pyrenophora tritici-repentis* in Oklahoma

INTRODUCTION

Pyrenophora tritici-repentis (PTR) (anamorph: *Drechslera tritici-repentis*) is the causal agent of tan spot, a serious foliar disease of wheat that has increased worldwide since the 1970s (Strelkov and Lamari 2003). Changes in agricultural practices toward reduced tillage or no-till are the main reason behind the increase in the incidence of this disease in wheat producing regions (Moreno et al. 2008; Singh and Hughes 2006). PTR is a homothallic fungus able to reproduce asexually and sexually, this allows the pathogen to recombine and produce different races (Aghamiri et al. 2015). Furthermore, this pathogen shows great variability in its genome and virulence (Moreno et al. 2008).

Knowledge of the pathogenic variation, genetic diversity and population structure of plant pathogens is a key component of breeding strategies to improve and develop cultivars with resistance against plant diseases. Studies have shown that genetic variability among populations of fungal pathogens can be due to many different factors including mutations, migration, mating system, population size and selection (Leisová et al. 2008). Currently, several molecular markers are available to analyze the genetic variability of plant pathogenic fungal populations, such as restriction fragment length

polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified length polymorphism (AFLP), microsatellites and sequencing based methods (Moreno et al. 2008).

Microsatellites, including simple sequence repeats (SSRs) and inter-simple sequence repeats (ISSR), have proven to be effective markers to study the genetic variability of PTR (Aboukhaddour et al. 2011; Aghamiri et al. 2015; Gurung et al. 2013; Moreno et al. 2008). SSRs are short motifs of up to six nucleotides in length that are repeated in tandem throughout the genome. SSR markers have been widely used in population studies because they are highly polymorphic, locus specific, codominant, and mostly selectively neutral (Milgroom 2015). Polymorphisms using SSRs can be detected by PCR amplification using locus-specific primers, which can be labeled with fluorescent dyes to determine the allelic fragment size variation by capillary electrophoresis (Aboukhaddour et al. 2011; Milgroom 2015).

Studies of the population structure and migration patterns of PTR using SSR markers on isolates from different continents have shown a high genetic diversity and moderate to high population differentiation (Gurung et al. 2013). In a study using isolates from North America, South America and Europe, population genetic analysis of PTR demonstrated no genetic grouping of fungal races and no groupings between geographic locations across different regions (Friesen et al. 2005). Similar results were found when studying the race structure of PTR in Canada, where a diverse population of isolates was found and no correlation between DNA polymorphisms and geographical distribution was observed (Singh and Hughes 2006).

In the Northern Great Plains, studies of genetic diversity of PTR have demonstrated that this fungal pathogen forms a diverse population in wheat and noncereal grasses when 270 single-spore isolates of PTR and 10 noncereal grasses were analyzed (Ali and Francl 2003). However, little is known about the population structure of PTR in Oklahoma. Therefore, the objective of this study was to determine the population structure of PTR in no-till wheat- producing areas of Oklahoma since a better understanding of the genetic variation is relevant to determine the pathogen's dispersal mechanisms and consequently improve management practices in the state.

MATERIALS AND METHODS

Isolates

To analyze the population structure of PTR across the main wheat producing counties in Oklahoma, a set of 180 PTR single spore isolates was selected from isolates collected from ten different no-till wheat fields (A, B, C, F, G, H, I, J, L, M) in 2016 (Figure 4.1). From fields A, F, I, and J, 30 samples per field were selected and from fields B, C, G, H, L, M, 10 isolates per field were selected. To analyze the population structure over two cropping seasons, four representative, no-till wheat fields (A, F, I and J) previously sampled in 2016, were sampled again in 2017 (circled fields, Figure 4.1). Forty isolates from each field (30 isolates from 2016 and 10 isolates from 2017) were selected (160 isolates in total). All isolates (n=220) were analyzed using simple sequence repeats (SSRs).

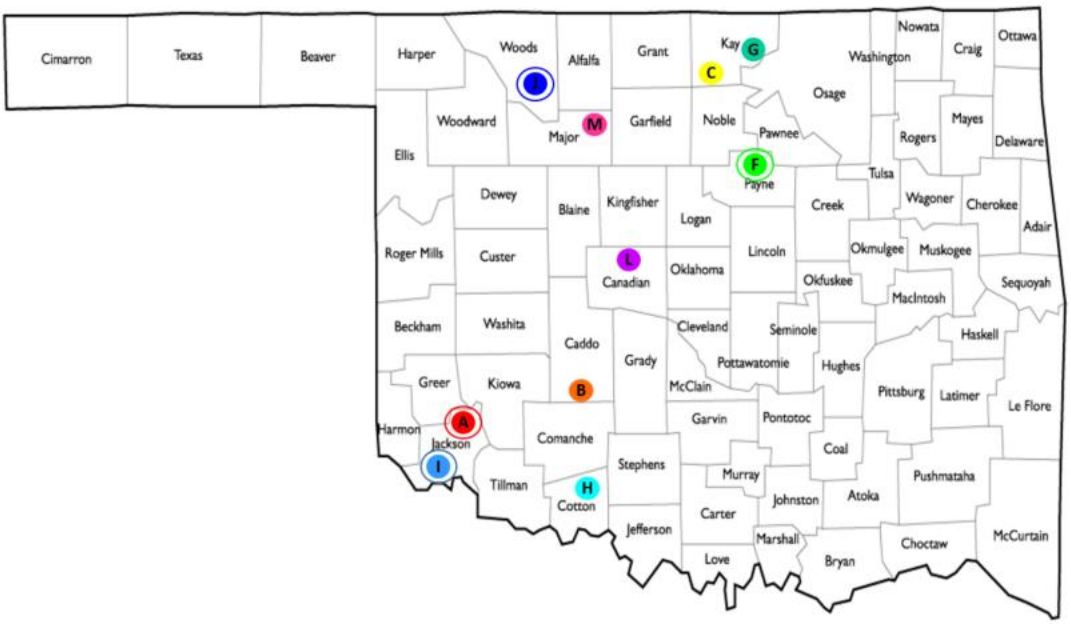

Figure 4.1. The population structure of PTR in Oklahoma was examined by comparing SSR allelic information and genetic differentiation among ten field subpopulations sampled during 2016 (A, B, C, F, G, H, J, L, M), and I, and four during 2017 (A, F, I and J) represented with a double circle.

Simple Sequence Repeats (SSR) analysis

Previously reported SSR primers (Gurung et al. 2013) were assessed for polymorphisms on a subset of PTR DNA samples (n=11) from different years collected in Oklahoma. Polymorphic loci primers (loci with * in Table 4.1) were used to generate SSR fingerprints for the entire 2016 and 2017 isolates (Table 4.1). SSR fingerprinting was performed according to the original protocol (Gurung et al. 2013). Briefly, individual PCR amplifications were performed for each microsatellite locus in 25 µl reactions, using 35 ng of template genomic DNA, 12.5 µl of 2X GoTaq Green Master Mix (Promega, Madison, WI), 6 µl of nuclease-free water, and 1 µl of each 5 µM primer. PCR conditions were as follows: 94 °C for 5 min, followed by 32 cycles of 95 °C for 30 s, 55 °C for 20 s,

72 °C for 30 s, with a final extension at 72 °C for 10 min. PCRs were performed in a MJR PTC-200 thermal cycler (Marshall Scientific, Hampton, NH). Electrophoresis of PCR products was performed on EtBr stained 2% agarose gels at 100V for 1 hour in TAE buffer. DNA fragments were visualized as described on Chapter III.

Table 4.1. Simple sequence repeats (SSR) loci, repeat motifs, expected fragment sizes, and locus specific primer sequences for PTR (Gurung et al. 2013) used in this study.

LOCUS	REPEAT MOTIF	LENGTH (bp) IN REFERENCE GENOME SEQUENCE	PRIMER SEQUENCES 5'-3'
PtrSSR01*	(AAC)32	286	F: TTTGGGAGATGGGGGAAA
			R: TTGCGTCTGTGCGACATG
PtrSSR03*	(AG)25	214	F: ACGTCCCTGCTTACGGATGT
			R: ATACCAAGCTTCTCCGCCTCT
PtrSSR05*	(AAG)26	241	F: GCGTGATCTCGCCACATATTA
			R: CGACGTCCTTCACAGGATTTA
PtrSSR06*	(AAG)17	209	F: TGCATTTGTGGTGCAAGATC
			R: AGAAGCCTTGGCCATTTTCA
PtrSSR07	(AGT)11ACGTTGT(AGT)12	238	F: ATCCACCTCCGTTGCAGTT
			R: CTGATTTCACCGCGAAAACA
PtrSSR09*	(AGT)31	285	F: CCACGCTCATCACTTTGTCTA
			R: CCACTTGATTGAGTTTTGCG
PtrSSR12	(AAG)18	256	F: AGAGGTGTCGACTAGCGTTT
			R: GGCTTAATTTTAAGCGCGTG
PtrSSR13*	(AAG)23	329	F: TCGTGGGTATAAAACGGCTCT
			R: TTCGGCGGCTTCATATTACT
PtrSSR14	(AAT)20	213	F: TAGAGCCTGCCGAGATTGTTT
			R: GCCTTCGGCCAAAGTCATA
PtrSSR15	(AAG)37	265	F: CGCCAACATAGTTGCGAATT
			R: TCCGCCTCTGTAGGTGTTAAA
PtrSSR16*	(AGT)37	326	F: TTACGATGCGCTCACACTAGA
			R: ATGGCCCGCTTGTAGTCTTTA
PtrSSR18	(AAG)10	231	F: TGTGGTGGATTCCCATTTCA
			R: CGAAGTAGAGTAGAGGGCCT

* Polymorphic loci in this study.

M13-tailed forward primers were used to label amplicons in a subsequent labeling PCRs with, M13 primers fluorescently 5' labeled with either of the fluorescent dyes fluorescein (6-FAM) or hexachlorofluorescein (HEX), for fragment sizing using an automated sequencer (Barkley et al. 2007). Briefly, the labelling PCRs were performed in 20 µl reactions that consisted of 0.04 µM of the M13-tailed forward primer, 0.16 µM of the reverse primer, 0.16 µM of the labelled M-13 primer (5'-6-FAM or 5'-HEX, IDT, Skokie, IL), 9 µl of 2X GoTaq Green Master Mix (Promega Madison, WI), 2 µl of nuclease-free water and 1µl of the PCR product obtained in a previous SSR reaction as template. PCRs were performed in a MJR PTC-200 thermal cycler (Marshall Scientific, Hampton, NH) with the following cycling program: 94°C for 5 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 45 s, 72 °C for 45 s, followed by 8 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min (Barkley et al. 2007). Amplicons were resolved electrophoretically on agarose gels to confirm amplification as described above.

Labelled PCR products were sized using capillary electrophoresis in an ABI 3700 automated sequencer (LabX, Midland, ON, Canada). Before sizing, labelled PCRs were diluted in PCR water to obtain optimum fragment sizing results. The rates of dilution varied between PCR products (1:10 to 1:80), and the optimum dilution ratios were determined empirically. Samples were prepared for sizing as follows: 1µl of each diluted PCR product was combined with 9 µl of formamide and 0.5 of the LIZ600 size standard v2.0 (ThermoFisher Scientific, Waltham, MA), and sent for fragment size analysis at OSU's Biochemistry and Molecular Biology Recombinant DNA and Protein Core Facility. The fragment sizes were scored in base pairs (bp) using Peak Scanner software

v1.0, and then entered into genotypic matrix (allelic databases) in Excel 2016 (Microsoft, Redmond, WA).

Data analysis

Statistical analyses were conducted using GenAlex 6.502 (Peakall and Smouse 2012) and PoppR 2.0. (Kamvar et al. 2015) using the SSR genotypic matrix constructed in Excel. A clonal correction was performed to ensure that only one individual per multilocus genotype was represented in the population. Isolates with the same multilocus genotype were considered clones. Multilocus genotypes were imported to R studio and analyzed using the PoppR package (Kamvar et al. 2015). Data analysis was performed on 180 PTR isolates from 2016, and a second analysis was performed on 160 PTR isolates collected in 2016 and 2017.

The unweighted pair group method with arithmetic means algorithm (UPGMA) using Nei's genetic distances and minimum spanning network (MSN) based on Bruvo's genetic distance for microsatellite loci were used to identify genetic relationships between isolates. A genotype accumulation curve was generated to determine the number of loci necessary to discriminate between individuals on the population. Subpopulations were defined by field of origin to determine if population structure could be explained by geographic distribution in Oklahoma.

To determine population structure of PTR in Oklahoma, the genetic differentiation among subpopulations was examined using analysis of molecular variance (AMOVA) and the F_{ST} statistic (genetic differentiation among population index) among PTR populations. ϕPT is a population differentiation statistic generated by AMOVA that

is similar to F_{ST}. F_{ST} values from 0 to 0.05 indicate low genetic differentiation; F_{ST} values from 0.05 to 0.15, moderate genetic differentiation; F_{ST} values from 0.15 to 0.25, great genetic differentiation; and F_{ST} values higher than 0.25, very great genetic differentiation (Wright 1978). ϕPT and *p* values were obtained with 999 permutations. Pairwise population F_{ST} analyses were performed to compare genetic differentiation between field subpopulations.

RESULTS

Seven of the 12 SSR markers tested on a subset of isolates were polymorphic and were used to analyze the ten 2016 and the four 2016-2017 PTR subpopulations in Oklahoma (Table 4.1). Population structure analysis using AMOVA on 180 PTR single spore isolates from 2016 showed low genetic differentiation among populations based on their geographic distribution, ϕPT =0.028. AMOVA analysis also revealed that for the 180 PTR isolates most of the genetic variation was found within populations (97% of the total variance), while genetic variation among populations represented a small part of the total (3% of the total variance) (Table 4.2 and Figure 4.2). Pairwise F_{ST} comparisons between field subpopulations showed moderate genetic differentiation between six populations, where C was the most differentiated subpopulation (Table 4.3) suggesting limited dispersion of inoculum from field C (Scheiber, Kay Co.) towards the other Oklahoma locations in this study.

Table 4.2. Analysis of molecular variance (AMOVA) of seven SSR markers for 180 PTR isolates from ten populations in 2016. df: degree of freedom; SS: sums of squared deviations.

Source	df	SS	Estimated Variance	Proportion of variance components (%)
Among Populations	9	39.061	0.083	3%
Within Populations	170	492.800	2.899	97%
Total	179	531.861	2.982	100%
PhiPT			0.028	

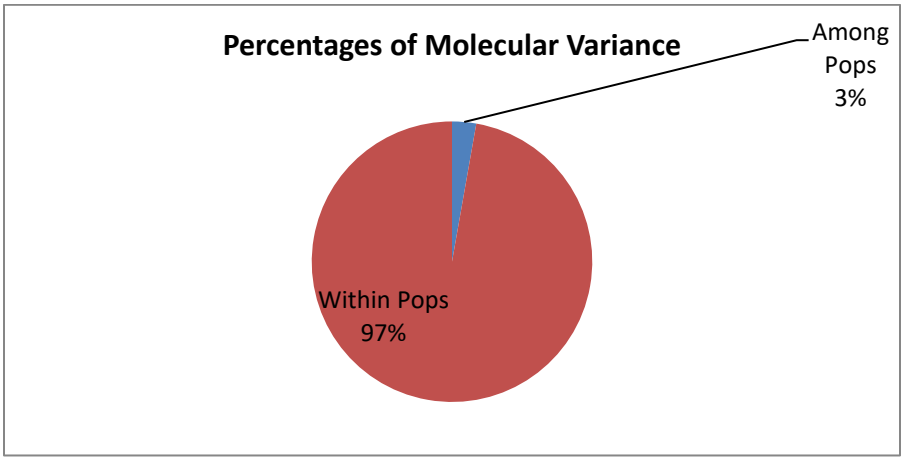

Figure 4.2. Percentages of molecular variance within and among PTR populations in Oklahoma on 2016.

Table 4.3. Pairwise F_{ST} values from ten no-till wheat field subpopulations obtained in 2016. Values of $F_{ST} > 0.05$ are highlighted, showing moderate genetic differentiation.

A	B	C	F	G	H	I	J	L	M	
0.000										**A**
0.013	0.000									**B**
0.044	0.070	0.000								**C**
0.018	0.007	0.054	0.000							**F**
0.004	0.008	0.042	0.035	0.000						**G**
0.006	0.029	0.059	0.044	0.021	0.000					**H**
0.013	0.003	0.073	0.012	0.026	0.022	0.000				**I**
0.025	0.035	0.096	0.028	0.027	0.058	0.043	0.000			**J**
0.000	0.034	0.000	0.015	0.005	0.017	0.026	0.040	0.000		**L**
0.015	0.042	0.032	0.026	0.036	0.025	0.028	0.068	0.000	0.000	**M**

The genotype accumulation curve demonstrated that the number of SSR markers used in this study was sufficient to explain the population structure of the PTR populations analyzed since the boxplot reaches 100% (Figure 4.3).

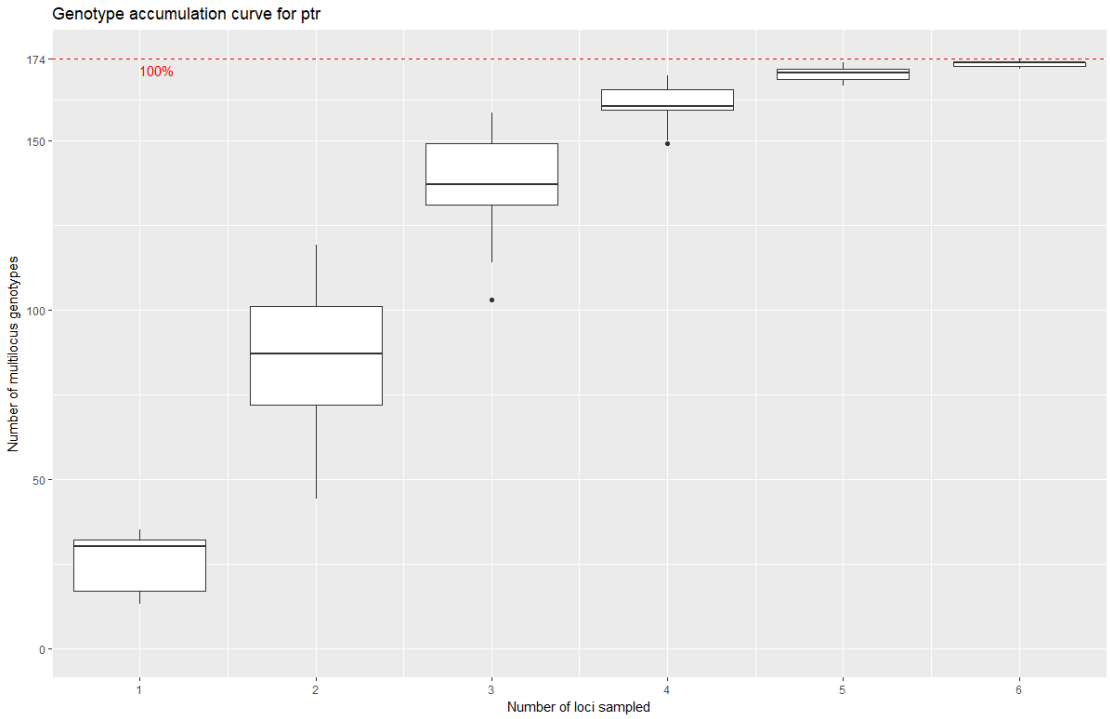

Figure 4.3 Genotype accumulation curve for SSR loci used to analyze PTR populations from 2016

In total, 174 multilocus genotypes were found among 180 PTR isolates from 2016 (Figure 4.4). The genotypic relationships determined by UPGMA analysis of the 180 PTR isolates revealed that individuals from different fields were distributed across all the clusters with no consistent pattern according to their geographic origin, supporting the low genetic differentiation identified by AMOVA. Still, small clusters of individuals belonging to the same field were observed, suggesting closely related genotypes could be found in the same fields, which can be explained by local inoculum dissemination.

Minimum spanning network (MSN) analysis revealed similar results to those in the UPGMA (Figure 4.5). In the MSN, nodes represent individual multilocus genotypes and node size reflects the number of individuals with the same genotype. Nodes with thicker and darker lines are more closely related, while nodes with lighter and thinner lines are more distantly related. As shown in Figure 4.5. the great majority of PTR isolates from 2016 presented unique genotypes. However, five clonal genotypes were found. Fields F, I and J showed one local clonal genotype each with two isolates (I and J) or three isolates (F) per clone in each field. Two isolates with the same genotype were found in two distant fields (A and B) which demonstrated that long distance movement of inoculum occurred between fields in different counties in the 2016 cropping season.

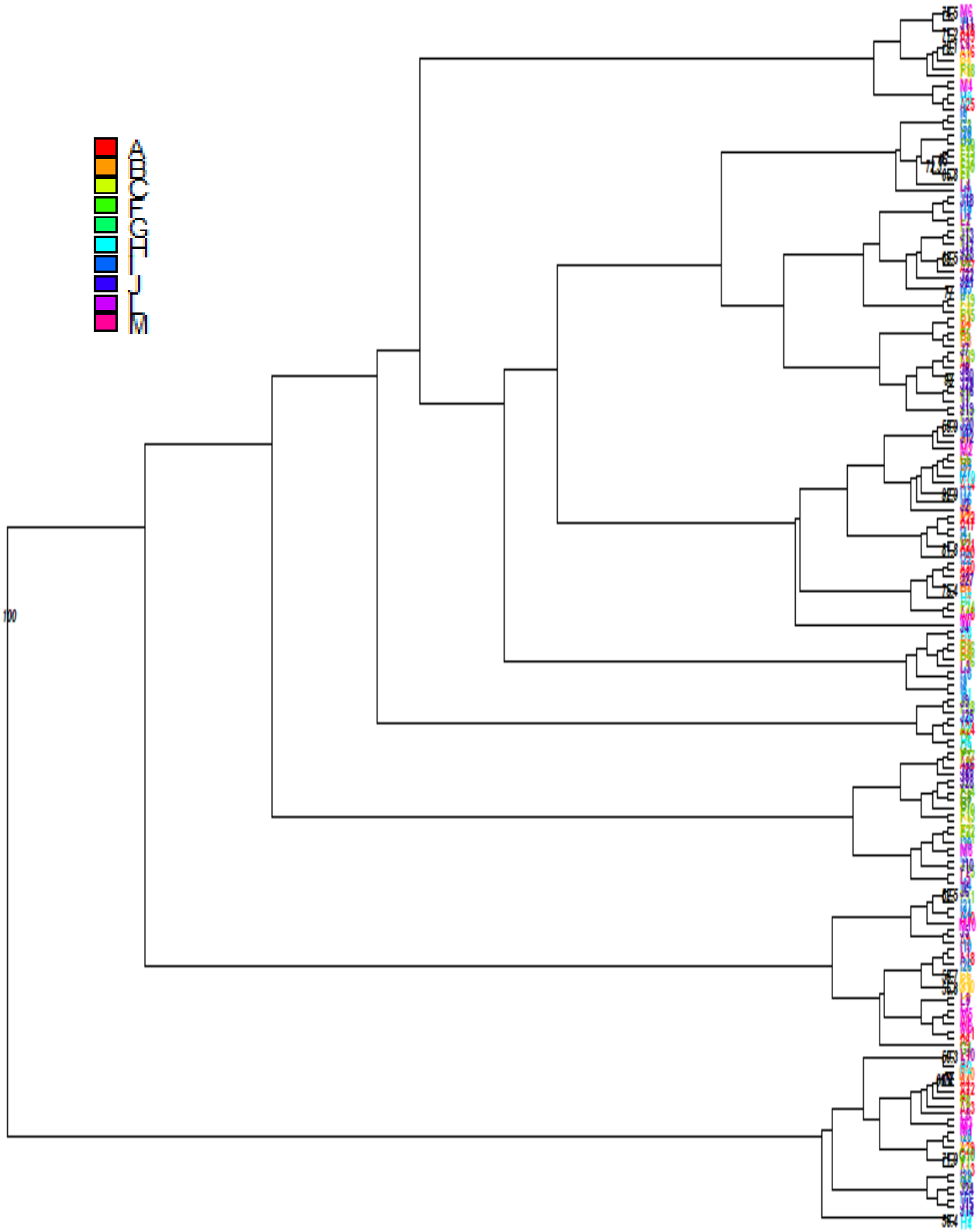

Figure 4.4. UPGMA dendrogram based on Nei's genetic distances among clone-corrected multilocus genotypes obtained with seven SSR markers on DNA from PTR isolates collected in 2016 from 10 no-till wheat fields in Oklahoma. Numbers on the branches show bootstrap supports greater than 50%.

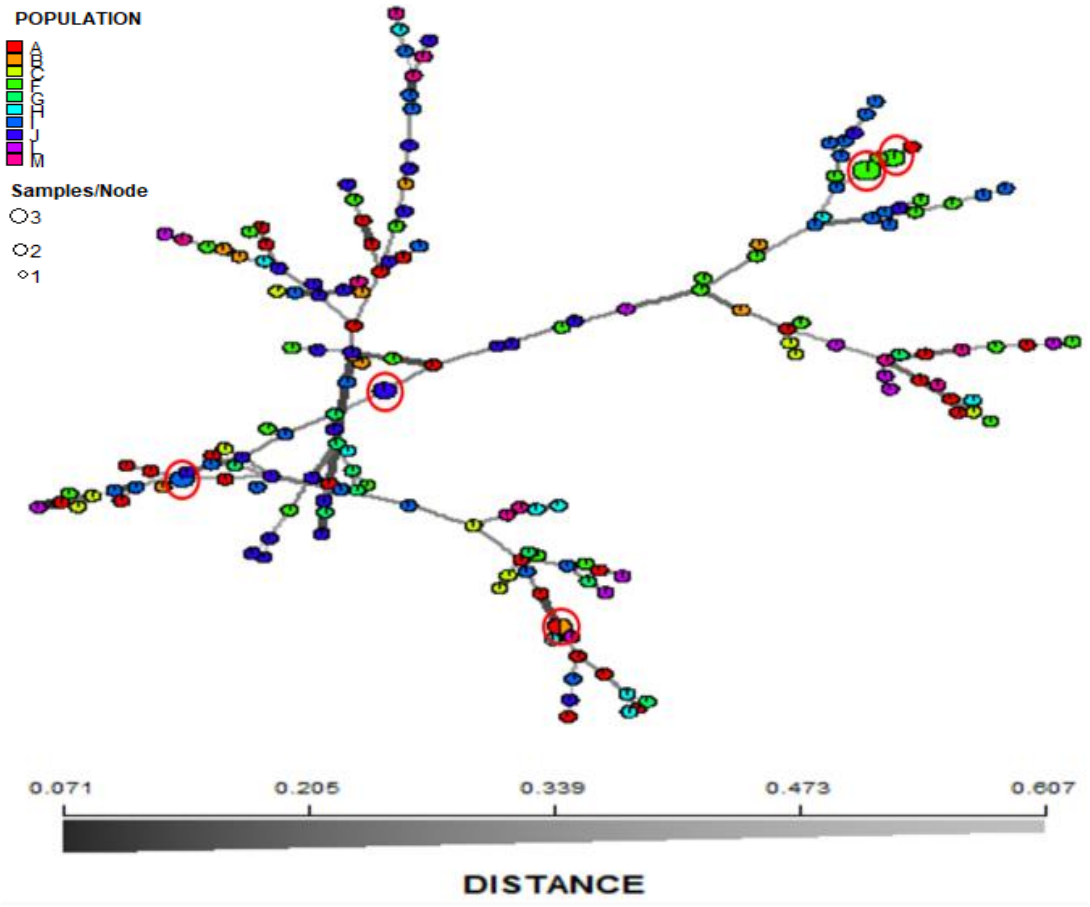

Figure 4.5. Minimum spanning network based on Bruvo's genetic distances among multilocus genotypes of PTR isolates collected from ten Oklahoma no-till wheat fields in 2016. Clonal genotypes with two or three individuals are highlighted with red circles.

Temporal population dynamics (2016-2017)

AMOVA based on the analysis of seven SSR loci from 160 single spore PTR isolates in eight annual subpopulations sampled from four fields in 2016 and 2017 showed a ϕPT value of 0.020 (*p*=0.001), indicating low genetic differentiation among subpopulations (Table 4.4). Most of the total molecular variance was observed within subpopulations (98%) and just a small proportion (2%) was among subpopulations (Table 4.4 and Figure 4.6). Of the 160 PTR isolates evaluated, 151 had unique multilocus SSR

genotypes. Pairwise F_{ST} comparisons between field subpopulations in two years, showed moderate genetic differentiation between two populations from 2017. Field F and I both from 2017 were the most differentiated subpopulations (Table 4.5) suggesting limited dispersion of inoculum from these fields towards the other Oklahoma locations during 2017. Again, the number of SSR loci markers used were sufficient since the genotype accumulation curve reached 100% (Figure 4.7).

Table 4.4. Analysis of molecular variance (AMOVA) of eight subpopulations using SSR genotypes of 160 PTR isolates collected from four fields in 2016 and 2017. df: degree of freedom; SS: sums of squared deviations.

Source	df	SS	Estimated Variance	Proportion of variance components (%)
Among Populations	3	15.869	0.060	2%
Within Populations	156	449.350	2.880	98%
Total	159	465.219	2.941	100%
PhiPT			0.020	

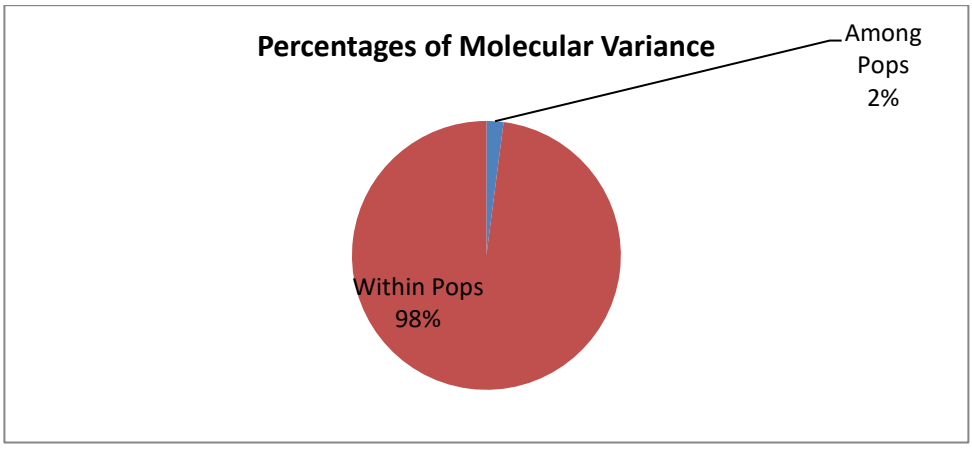

Figure 4.6. Percentages of molecular variance within PTR populations and among PTR populations from Oklahoma in 2016 and 2017.

Table 4.5. Pairwise F_{ST} values from four no-till wheat fields subpopulations obtained in 2016 and 2017. Values of F_{ST} <0.05 are highlighted, showing moderate genetic differentiation.

A.16	A.17	F.16	F.17	I.16	I.17	J.16	J.17	
0.000								**A.16**
0.009	0.000							**A.17**
0.018	0.015	0.000						**F.16**
0.028	0.021	0.039	0.000					**F.17**
0.013	0.034	0.012	0.038	0.000				**I.16**
0.000	0.028	0.021	0.015	0.015	0.000			**I.17**
0.025	0.008	0.028	0.051	0.043	0.061	0.000		**J.16**
0.030	0.012	0.005	0.099	0.031	0.072	0.000	0.000	**J.17**

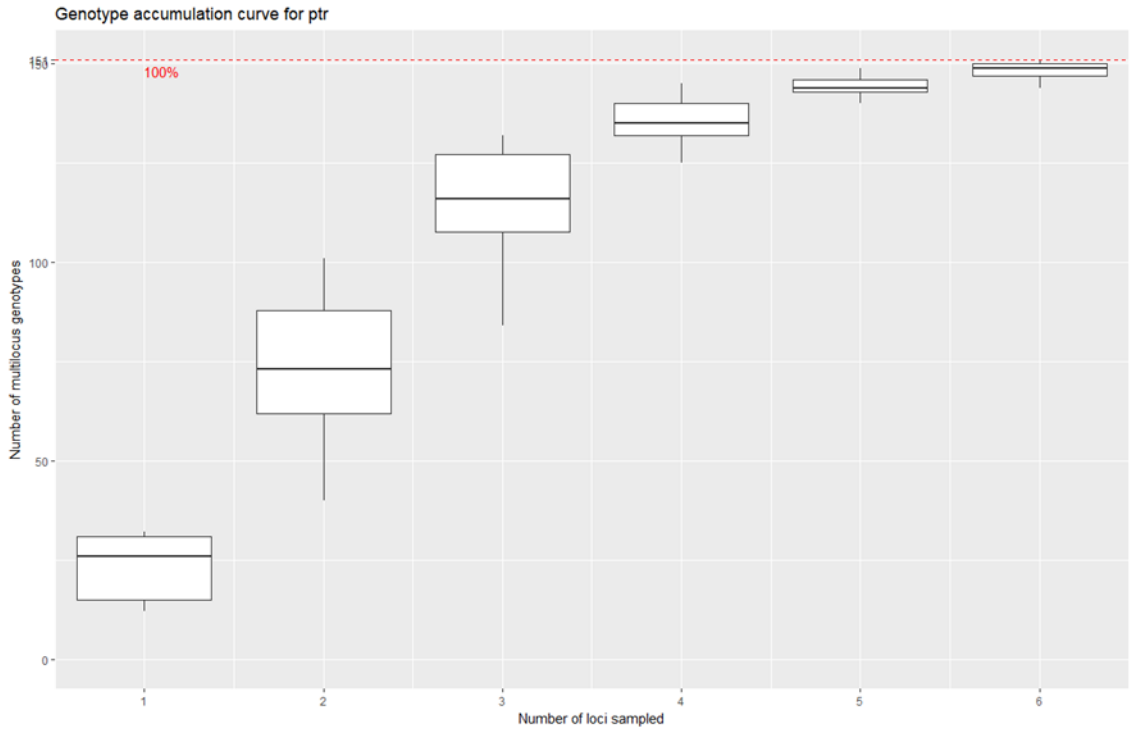

Figure 4.7. Genotype accumulation curve for PTR populations from 2016-2017 with seven SSR markers.

UPGMA analysis using Nei's genetic distances among the genotypes of 160 PTR isolates from four fields in 2016-2017 revealed that the majority of individuals were distributed throughout all the clusters and no groups corresponded with a single location

or year of sampling were observed (Figure 4.8). Several individual genotypes from the same fields were grouped together within each cluster. These results indicate that inoculum can be dispersed long distances, since closely related isolates were collected from distant counties.

MSN with the 160 PTR isolates from 2016-2017 supported the results of the UPGMA analysis (Figure 4.9). Although most PTR isolates had unique genotypes, several clonal genotypic clusters were found among individuals from the same field, from different years. In field F, three clonal genotypes were observed. The first clonal genotype was observed in two isolates collected in 2016. The second clonal genotype was observed in two isolates collected in 2017. And, the third clonal genotype was found in three isolates collected in 2016. Field I had a clonal genotype found in two isolates from 2016. Field J presented three clonal genotype clusters: a clonal genotype in two isolates from 2016, a second clonal genotype in two isolates from 2017, and a third clonal genotype with one isolate from 2016 and one isolate from 2017. These results supported the hypothesis that inoculum was locally distributed in each field, both in 2016 and 2017, and the inoculum persisted in the same field between cropping seasons. Furthermore, two isolates collected on different years had the same clonal genotype and were found in two different fields in the same county: one 2016 isolate from field I and one 2017 isolate from field A. This observation demonstrated that movement of PTR inoculum had occurred between two fields in the same county, and that genotypes observed in 2016 could reemerge at some distance in the same county during the 2017 cropping season (Figure 4.9).

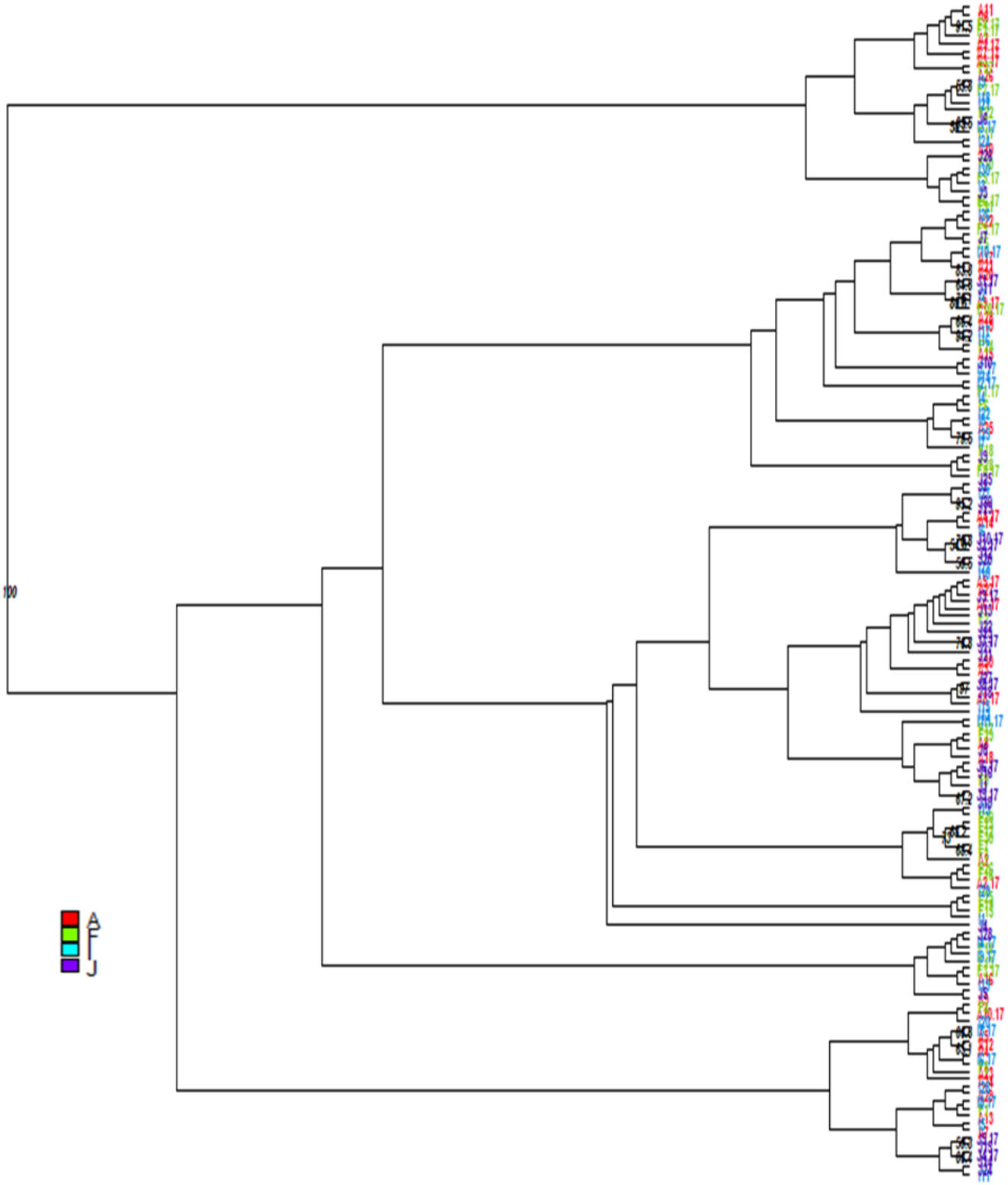

Figure 4.8. UPGMA dendrogram based on Nei's genetic distance on clone-corrected multilocus genotypes (MLGs) obtained with seven SSR markers, of PTR isolates collected from four no-till wheat fields in 2016-2017. Each isolate in this tree represents a unique MLG. Numbers on the branches show bootstrap supports greater than 50%.

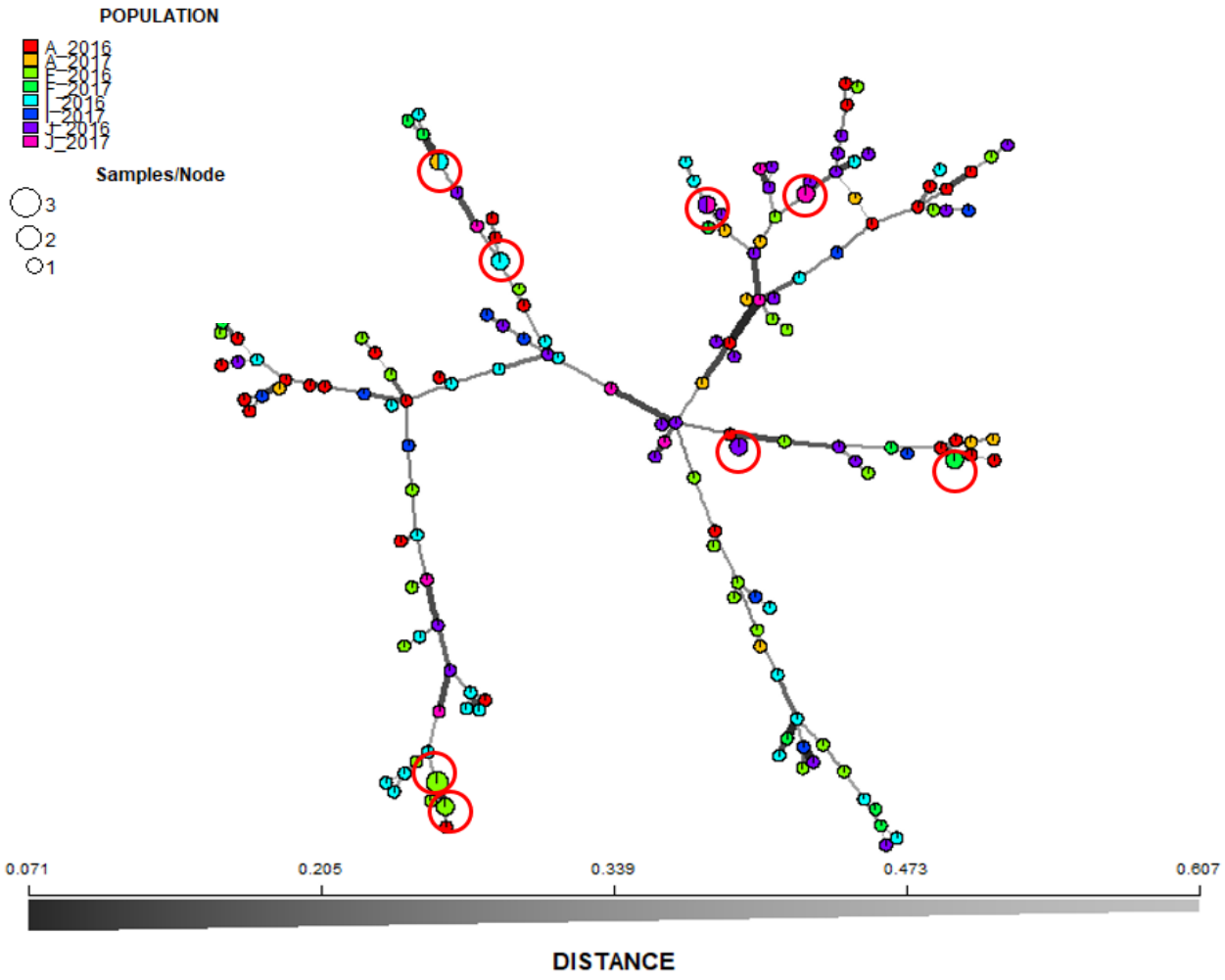

Figure 4.9. Minimum spanning network based on Bruvo's genetic distances among multilocus genotypes of PTR isolates collected from four Oklahoma fields in 2016 and 2017. Clonal genotypes are highlighted in red circles.

DISCUSSION

Different molecular methods have been used to study the population structure of *P. tritici-repentis*. SSRs have been considered among the most suitable molecular markers because of their locus-specificity, highly polymorphic nature and their higher mutation rates (Milgroom 2015; Mironenko et al. 2016). Seven SSR markers were used to examine isolate diversity and genotype relatedness, as well as the level of genetic

differentiation among PTR populations collected from different wheat fields over two cropping seasons. Seven of the microsatellites reported by Gurung et al. (2013) were highly informative for the study of PTR populations in Oklahoma.

According to AMOVA analysis, PTR populations across ten different counties in 2016 and four counties in 2017 had low genetic differentiation, implying frequent movement of inoculum between fields across counties. No consistent population structure defined by field of origin or year of collection was found. Two clustering methods, UPGMA and MSN, showed PTR populations were highly diverse, with most isolates presenting unique SSR genotypes. Nonetheless, several clonal genotypes were observed. In 2016, 97% of the multilocus genotypes were unique among 180 isolates, while comparisons of the 2016 and 2017 populations from four fields found 94% unique multilocus genotypes among 160 isolates. Most of the genetic variability was found within populations. These results indicate that the Oklahoma PTR population is highly diverse with extensive movement of inoculum across the state.

Genetic variability among fungal pathogens is mainly caused by mutation and recombination, however, migration and gene flow also play important roles in genetic variation of populations (Moreno et al. 2012). While mutation and recombination increase population differentiation, leading to population structure due to the limited exchange of new alleles between populations, migration and gene flow allow the movement of new alleles variants between populations, increasing the genetic diversity and homogeneity of populations (Milgroom 2015). High genetic variation has been previously found in PTR populations in several studies from different regions around the world (Benslimane et al. 2013; Friesen et al. 2005; Leisová et al. 2008; Santos et al.

2002; Singh and Hughes 2006). PTR is an airborne homothallic fungus capable of reproducing sexually and asexually in the field. Sexual recombination is probably the main reason for high levels of genetic variability observed in PTR populations, since recombination creates new combinations of alleles resulting in the appearance of new and unique genotypes (Aghamiri et al. 2015; Ciuffetti et al. 2014; Friesen et al. 2005; Gurung et al. 2013; Moreno et al. 2012). Asexual reproduction plays an important role in disease dissemination of clonal linages, with multiple cycles of conidia production taking place each season (Friesen et al. 2005; Leisová et al. 2008). Furthermore, PTR can be dispersed long distances by wind. Studies have shown that conidia can travel up to 200 km when environmental conditions are suitable (Francl 1997; Moreno et al. 2012). Additionally, PTR can be seed-borne, which allows the pathogen to travel even longer distances moved by humans. Sexual recombination, combined with long distance movement of air-borne and seed-borne inoculum, contribute to the high genetic diversity of PTR regardless of the geographic distribution (Aghamiri et al. 2015; Moreno et al. 2012).

The two cluster analyses performed, UPGMA and MSN based on genetic distances, show almost no grouping patterns defined by geographic origin or year of collection. However, small groups of closely related isolates were collected from the same field, which suggests that endemic inoculum plays an important role initiating tan spot epidemics. Furthermore, the appearance of clonal multilocus genotypes within fields in the same cropping season, suggests some local infections are caused by conidia (Halkett et al. 2005). The occurrence of clonal and closely related genotypes across fields in the same county or in distant counties was not unexpected, since the distances between

Oklahoma counties sampled were within the range of dispersion of PTR airborne inoculum (Adee and Pfender 1989; Oliver et al. 2008).

Field A (Altus) and field B (Apache) are both located in the southwestern part of the state and are only approximately 58 miles (93 km) apart. According to the Mesonet Climatological Data Summary, during March of 2016 in Altus, when samples were collected, southwesterly wind speeds averaged approximately 13.0 mph, with a highest speed of 48.7 mph. This could have increased the likelihood of spores traveling the short distance between fields (McPherson et al. 2007). The wind speed and direction, would have favored spores movement from Altus to Apache.

Comparison of the 2016 and 2017 populations from four fields yielded results similar to those observed in 2016 alone. AMOVA found low genetic differentiation among populations, and no clustering corresponding to geographic origin or year of sampling was observed. However, a few clonal genotypes were found locally, supporting the likelihood of asexual inoculum movement within the fields. Interestingly, a clonal genotype was found among isolates collected from two different fields in the same county in two different years. This result supports the hypothesis that inoculum not only moves between fields but can survive from one cropping season to the next. Field A (Altus) and Field I (Olustee) were also a short distance apart, with only approx. 8 miles (13 km) between them, hence, winds could have easily moved inoculum between those fields. As a necrotrophic fungus, PTR is capable of surviving saprotrophically and as protopseudothecia on the stubble left in the field at the end of the previous cropping season. Then ascospores produced in mature pseudothecia can serve as primary inoculum the following season. Consequently, our data further supports the pathogen surviving on

crop debris in no-till or reduced tillage wheat fields (Ciuffetti et al. 2014; Summerell and Burgess 1989). In the present study, all the sampled fields were no-till. Therefore, it is likely that the inoculum present in 2016 in Olustee could have persisted on debris in that field in Jackson County and then moved on prevailing southwesterly winds to surrounding fields, reaching the field in Altus in 2017. Based on these results, we can assume that the movement of inoculum between fields is common and constant, which can be mainly related to environmental conditions, although human activities may also play a role in inoculum dispersal. Moderate genetic differentiation was found mostly between field C (Scheiber, Kay County) and five other fields, suggesting that some inoculum from this field had limited distribution in Oklahoma. Subpopulations from two fields, L (Banner Road, Canadian County) and M (Lahoma, Major County), located southwest from Scheiber, and field G, also in Kay County (Kildare), were not significantly differentiated from the field C population, suggesting frequent exchange of inoculum between fields in those counties.

Overall, our results showed high levels of polymorphisms in PTR isolates but no population differentiation was found due to geographic origin or year of collection. Results also provided evidence of frequent movement of genetic material within fields and dispersal of inoculum to neighboring fields, as well as across counties; as well as survival of inoculum over two cropping seasons. This study provides a base line for a better understanding of PTR populations in Oklahoma and should help the development of management strategies to prevent and control tan spot. Future assessment of tan spot resistance in wheat breeding lines should include diverse isolates representing the predominant pathogen lineages in Oklahoma.

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CHAPTER V

EVALUATION OF THE PRODUCTION OF HOST-SELECTIVE TOXINS A AND B OF *Pyrenophora tritici-repentis*

INTRODUCTION

Tan spot of wheat caused by the fungus *Pyrenophora tritici-repentis* is a major foliar disease of global economic importance (Aghamiri et al. 2015). On susceptible hosts, *P. tritici-repentis* causes two very distinctive symptoms: tan necrosis or extensive chlorosis (Lamari and Strelkov 2010). In order to classify *P. tritici-repentis* isolates, a pathotype designation was originally suggested based on the ability of the pathogen to produce necrosis or chlorosis on differential wheat cultivars (Leisová et al. 2008). Later, a race classification system was established based on a *P. tritici-repentis* isolate's virulence variation on five wheat differentials cultivars (cvs.), Glenlea, Katepwa, Salamouni, and lines 6B365 and 6B662 (Andrie et al. 2007). Currently, *P. tritici-repentis* isolates can be grouped into eight races according to the symptoms observed on the host (Abdullah et al. 2017a). Race 1 induces necrosis on the differentials cvs. Glenlea and Katepwa, and chlorosis on line 6B365; race 2 causes necrosis on the differentials cvs. Glenlea and Katepwa, but does not produce chlorosis on line 6B365; race 3 causes chlorosis only on line 6B365; race 4 is considered avirulent, since it does not produce symptoms on any of the differential lines; race 5 induces chlorosis on the differential cv.

Katepwa and on line 6B662; race 6 combines the virulences of races 3 and 5; race 7 combines the virulences of races 2 and 5; while race 8 combines the virulences of races 2, 3 and 5 (Andrie et al. 2007; Effertz et al. 2002; Friesen et al. 2005).

Tan spot symptom development on susceptible wheat genotypes is related to the production of host selective toxins (HSTs). At present three peptide HSTs have been described for *P. tritici-repentis*, Ptr ToxA, Ptr ToxB and Ptr ToxC (Ali and Francl 2003). Ptr ToxA was the first HST to be described. It is a proteinaceous toxin responsible for inducing necrosis on susceptible cultivars (Ciuffetti et al. 2010; Manning et al. 2007). Ptr ToxA is a 13.2 kDa protein encoded by a single gene that affects photosystems I and II in the chloroplasts, and leads to the accumulation of reactive oxygen species (ROS) and causing cell death (Manning et al. 2009). Furthermore, several studies have suggested that Tox-A was likely obtained from *Parastagonospora nodorum*, another fungal wheat leaf spot pathogen, by horizontal transfer since the *ToxA* genes between the two species are highly similar (Ciuffetti et al. 2010; Friesen et al. 2006; Leisova-Svobodova et al. 2010).

Ptr ToxB is a chlorosis-inducing 6.5kDa protein HST present in multiple genomic copies and was found initially from a race 5 isolate (Strelkov et al. 2006). The site and mode of action of ToxB have not yet been characterized, but it could be similar to ToxA, as ToxB affects the chloroplast functions, resulting in photooxidation of the chlorophyll, and consequently disrupting photosynthesis (Figueroa et al. 2015; Pandelova et al. 2012). One unique feature of Ptr ToxB is the occurrence of multiple nontoxic homologs, denominated as *toxb*, in non-pathogenic isolates (Figueroa et al. 2015; Nyarko et al. 2014). These ToxB homologs have been found in race 3 and 4 meaning that there are

isolates of *P. tritici-repentis* that carry the *ToxB* gene without producing the toxin. The reason for this is still unclear. (Moreno et al. 2012; Strelkov et al. 2006).

Ptr ToxC is a HST that induces chlorosis, but on wheat differential cultivars and lines insensitive to Ptr ToxB. Tox-C appears to be a non-ionic, polar, and low molecular weight molecule (Abdullah et al. 2017b; Lamari and Strelkov 2010); however, little is known about this toxin. Although it has not been purified or characterized, it is still considered an important pathogenicity factor (Lamari and Strelkov 2010). Ptr ToxA is produced by races 1, 2, 7 and 8; Ptr ToxB is produced by races 5, 6, 7 and 8; while Ptr ToxC is produced by races 1, 3, 6 and 8 (Friesen et al. 2005).

Oklahoma is one of the major winter wheat producing states where tan spot has become a serious concern for wheat producers, due to adoption of reduce tillage or no-till practices, which increase crop debris that can harbor inoculum. Consequently, the characterization and evaluation of the HSTs is essential to develop tan spot management strategies including durable resistant varieties. Therefore, the objectives of this study were to analyze *P. tritici-repentis* isolates from 2016 and 2017 for the presence or absence of *ToxA* and *ToxB* genes, and to phenotype the pathogen races present in Oklahoma using differential wheat cvs. and lines.

MATERIALS AND METHODS

Genotyping of Tox A and Tox B

The presence of HST genes *Ptr ToxA* and *Ptr ToxB* in 311 *P.tritici-repentis* (PTR) isolates collected in 2016 from 13 no-till wheat fields in Oklahoma and 78 PTR isolates collected in 2017 from four no-till wheat fields previously sampled in 2016 were

evaluated. PTR HST gene detection was performed with PCR using previously reported primers. For PCR amplification of *Ptr ToxA*, primers TA51F (5′-GCGTTCTATCCTCGTACTTC-3′) and TA52R (5′-GCATTCTCCAATTTTCACG-3′) were used, generating a 591 bp amplicon (Andrie et al. 2007). Primers TB71F (5′-GCTACTTGCTGTGGCTATC-3′) and TB6R (5′-ACGTCCTCCACTTTGCACACTCTC-3′) were used to amplify *Ptr ToxB*, producing a 243 bp amplicon (Andrie et al. 2007). PCR reactions for both sets of primers (TA51F-TA52R and TB71F-TB6R) were performed in 25 µl reactions, using 70 ng of template DNA, 12.5 µl of 2X GoTaq Green Master Mix (Promega, Madison, WI), 6.5 µl of nuclease-free water, and 2 µl of each 5 µM primer.

The PCR program used for both sets of primers was 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 57°C for 30s, 72°C for 1 min; with a final extension at 72°C for 10 min. PCR reactions were performed in a PTC-200 thermal cycler (Marshall Scientific, Hampton, NH). Electrophoresis of PCR products was performed on EtBr-stained, 2% agarose gels run at 100V for 1 hour in TAE buffer. DNA fragments were observed in a GelDocIt Imagining System and analyzed using VisionWorks LS software (UVP, Upland, CA).

Phenotyping Characterization

Plant material

Wheat seedlings from five differential lines (Glenlea, Katepwa, 6B662, 6B365 and Salamouni) were used for the identification of PTR races based on leaf symptom produced after conidial inoculation. Wheat seeds were planted in Seed Germination mix

BM2 (Berger, Canada, QC) in small plastic containers. Two seeds were placed in each container with soil and plants were grown up to the third leaf stage in a growth chamber, with conditions set at 12h of light at 25°C and 12h of dark at 20°C. Plants were watered daily, and fertilized once, seven days post planting, with ~4 g L^{-1} Peters 20-20-20 general purpose fertilizer (The Scotts Co., Marysville, OH) and ~1 g L^{-1} VANSIL® W-20 (Vanderbilt Minerals. LLC, Gouverneur, NY).

Race phenotyping using conidia suspensions

For whole plant inoculation, five PTR single-spore isolates were selected from the 2016 and 2017 collections, based on the presence or absence of the *ToxA* and *ToxB* genes (Table 5.1). PTR isolates were cultured on SNA media (0.1% KH_2PO_4, 0.1% KNO_3, 0.05% $MgSO_4$ $7H_2O$, 0.05% KCl, 0.02% glucose, 0.02% sucrose, 2% agar) for seven days and then subcultured to clarified V8 media (1.63% V8 Juice, 0.163% calcium carbonate, 1.9 % agar) and incubated at room temperature in the dark for 5 to 7 days. To induce spore production, 1 ml of sterile water was added to each culture and matted down using a spreader. Next, the fungal cultures were incubated at room temperature under fluorescents lights for 24h to induce conidiophores, after which cultures were incubated at 16°C in the dark for 24h to induce conidia production. Conidia were suspended in 1000 µl of sterile water with a spreader and the spore suspension decanted into a tube. Prior to inoculation, conidia were counted using a hemocytometer and microscope and each spore suspension adjusted to ~1,500 conidia/ml in a volume of 25 ml of water. Each spore suspension was applied directly to leaves using a spray bottle, targeting the second and third leaf. Plants were then placed in a dew chamber at 100% humidity to induce

spore germination for 48h. Inoculated plants were incubated for seven days in a growth chamber with 12 h photoperiod, 25°C during the light cycle and at 20°C during the dark cycle. Each isolate was inoculated onto eight plants of each differential line per repetition and each assay was repeated three times. Symptoms on the differentials lines were assessed seven days post inoculation and scored according to the presence or absence of chlorosis (C), necrosis (N), resistance (R, small hypersensitive response necrosis) or no symptoms (NS, green leaves without lesions) (Figure 5.1). The symptoms were then compared to the scheme proposed by Andrie et al. (2007) for race classification (Table 5.2).

Table 5.1. PTR isolates used for host selective toxin production phenotyping.

Isolate Number	Field	Code	Year	*Tox A* *	*Tox B* *
1	I	K3-1NC-1	2016	+	−
2	J	L5-1NC-1	2016	−	−
3	A	A2-1NC-1	2017	+	−
4	I	K1-2NC-4	2017	+	−
5	J	L4-2NC-5	2017	+	−

**ToxA* and *ToxB* columns represent the presence (+) or absence (−) of the toxin genes

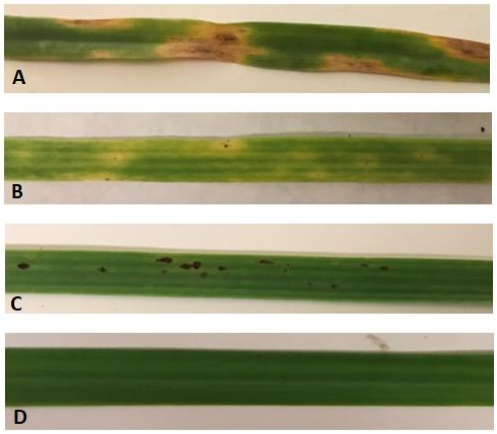

Figure 5.1. Tan spot symptoms on differential wheat lines seven dpi. A. Necrosis (N) on cultivar Glenlea. B. Chlorosis (C) on line 6B365. C. Hypersensitive response (R) on cultivar Salamouni. D. No symptoms (NS).

Table 5.2. Race structure of *Pyrenophora tritici-repentis* (Andrie et al. 2007)

	Wheat Differentials					
Race	Glenlea	Katepwa	6B662	6B365	Salamouni	Auburn
1	N (Tox A)	N (Tox A)	R	C (Tox C)	R	R
2	N (Tox A)	N (Tox A)	R	R	R	R
3	R	R	R	C (Tox C)	R	R
4	R	R	R	R	R	R
5	R	C (Tox B)	C (Tox B)	R	R	R
6	R	C (Tox B)	C (Tox B)	C (Tox C)	R	NT
7	N (Tox A)	N (Tox A) C (Tox B)	C (Tox B)	R	R	NT
8	N (Tox A)	N (Tox A) C (Tox B)	C (Tox B)	C (Tox C)	R	NT

N = Necrosis, C = Chlorosis, R = Resistance, ToxA = presence of *ToxA* and production of Ptr ToxA, ToxB = presence of *ToxB* and production of Ptr ToxB, ToxC = production of Ptr ToxC as evidenced by symptoms, and NT = isolates of race 6, 7 and 8 were not available for inoculation.

Race phenotyping culture filtrate infiltration

A second method using presumed HSTs in culture filtrates of PTR isolates was assessed. Culture conditions for PTR were followed as described by (Zhang et al. 1997) with minor modifications. Briefly, 15 PTR isolates (Table 5.3) were grown in clarified V8 agar (1.63% V8 Juice, 0.163% calcium carbonate, 1.9 % agar) media for seven to ten days. Mycelia on ten 0.5 cm diameter plugs were transferred to 50 ml of potato dextrose broth (PDB), in a Magenta vessel GA-7 (Sigma-Aldrich, St. Louis, MO), and kept for seven days at room temperature. After seven days, PDB media was removed from mycelia in vessels and replaced with 50 ml of liquid Fries medium (Dhingra and Sinclair 1985) added to each vessel. Cultures were kept in darkness at room temperature for 28 days to induce toxin production. Culture filtrates were recovered from vessels and 0.001% of Silwet® L-77 (Helena Agri-enterprises, LLC, Collierville, TN) was added prior to plant infiltration.

Plants were grown as previously described. In each plastic cone three seeds were planted. A total of six plants were inoculated per isolate with only one repetition. The culture filtrates with Silwet were infiltrated directly into the leaves with a plastic transfer pipette, until 2-3 cm of the second and third leaf were infiltrated. With a nontoxic permanent marker, the border of the infiltrated area was marked. Plants were kept in the growth chamber for four days with a 12 h photoperiod at 25°C during the day (light) and 20°C during the night (dark). Plants were scored as sensitive or insensitive based on the presence or absence of symptoms, four days post-infiltration. The observations were compared to the scheme proposed by Andrie et al. (2007) to determine PTR races.

Table 5.3. PTR isolates used for host selective toxin production using toxin extract infiltration.

Isolate Number	Field	Code	Year	*Tox A*	*Tox B*
1	A	A2-1NC-1	2017	+	−
2	A	A2-4NC-1	2017	+	−
3	F	H1-3NC-3	2017	+	−
4	F	H4-2NC-6	2016	+	−
5	I	K1-2NC-4	2017	+	−
6	I	K3-1NC-1	2016	+	−
7	I	K1-1NC-5	2016	+	−
8	J	L3-2NC-2	2017	+	−
9	J	L4-2NC-5	2017	+	−
10	A	A5-5NC-2	2016	−	−
11	F	H1-3NC-2	2016	−	−
12	F	H3-5NC-3	2017	−	−
13	I	K2-2NC-3	2016	−	−
14	I	K3-2NC-1	2017	−	−
15	J	L5-1NC-1	2016	−	−

ToxA and *ToxB* columns represent the presence (+) or absence (−) of the toxin genes

RESULTS

The production of HSTs by PTR isolates was determined by genotypic and phenotypic characterization.

HST Genotyping

A total of 389 PTR isolates from 2016 and 2017 were evaluated to identify the presence or absence of the *ToxA* and *ToxB* genes. Of a total of 311 PTR collected in 2016 isolates tested, 295 (94.85%) PTR isolates carried the *ToxA* gene (Figure 5.2) and 16 isolates (5.15%) lacked the *ToxA* gene. Similarly, out of 78 PTR isolates collected in 2017, 75 (96.15%) PTR isolates possessed the *ToxA* gene and only 3 isolates (3.85%) lacked the *ToxA* gene (Figure 5.3). None of the isolates evaluated, collected in either 2016 or 2017, were PCR-positive for the *ToxB* gene.

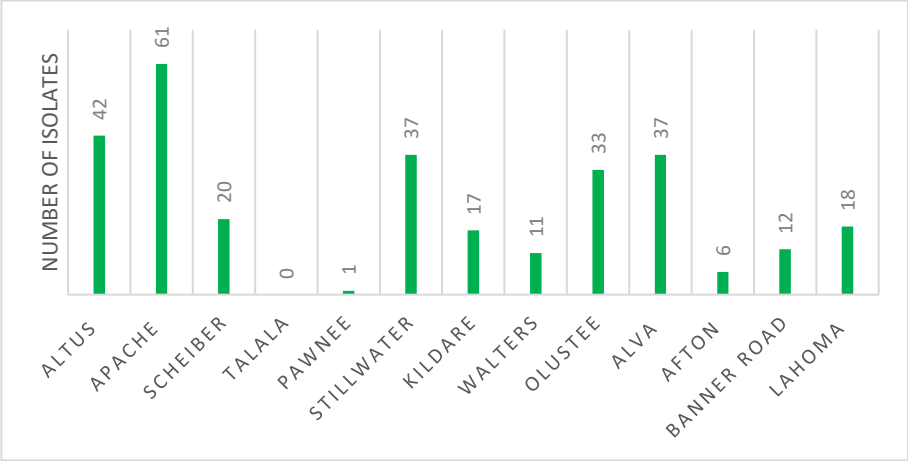

Figure 5.2. Number of PTR isolates possessing the *ToxA* gene from 13 no-till wheat fields from Oklahoma in 2016.

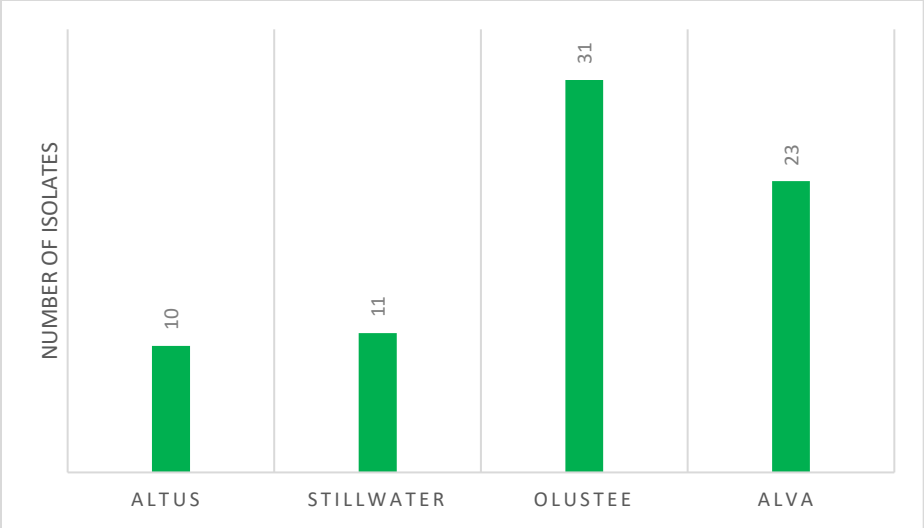

Figure 5.3. Number of PTR isolates possessing the *ToxA* gene from four no-till wheat fields from Oklahoma in 2017.

Phenotyping

Plant inoculation with conidia suspension

Four out of the five PTR isolates tested by inoculating differential wheat lines with conidia were identified as race 1 according to symptom production. Isolates classified as race 1 produced tan necroses on Glenlea and Katepwa, resistance HRs on line 6B662 and Salamouni, and chlorosis on line 6B365. Race identification was not possible for one of the isolates (L5-1NC-1) since symptom production did not correspond to any of the eight races previously described by Andrie et al. (2007).

Plant infiltration with culture filtrates

Race designation of the 15 PTR isolates tested was not possible using the infiltration method, because the results were ambiguous and inconsistent with the results as expected according to the original paper (Zhang et al. 1997).

DISCUSSION

Pyrenophora tritici-repentis (PTR) has a complex interaction with its wheat host, therefore the study of the physiological specialization is challenging (Andrie et al. 2007; Moreno et al. 2012). Currently, PTR isolates are segregated into eight races according to the symptoms produced on wheat differential lines by host-selective toxins (HST) produced by the fungus (Aboukhaddour et al. 2011). Ptr Tox A and Ptr Tox B are the best studied HSTs from PTR at present and specific primers have been developed to qualitatively assay the presence of corresponding genes (Andrie et al. 2007). Genotypic characterization of PTR isolates using *ToxA* and *ToxB* gene-specific primers demonstrated that 95% of the 2016 and 96% of the 2017 isolates examined possessed the *ToxA* gene. None of the isolates from 2016 or 2017 were positive for the presence of the *ToxB* gene. These results imply that in the state of Oklahoma there is a predominance of Tox-A-producing isolates responsible for tan spot in susceptible wheat cultivars. ToxA can be present in PTR races 1, 2, 7, and 8, which may be present in the state. However, the absence of the *ToxB* gene suggests the absence of races 5, 6, 7, and 8 (Andrie et al. 2007). Therefore, only races 1 and 2 may be present in Oklahoma.

Phenotypic characterization using inoculation of differential wheat cultivars with conidial suspensions was consistent with the previous expectations (Kader 2010), since four out of five isolates examined corresponded to race 1, according to the observed symptoms in five wheat differentials. The four isolates categorized as race 1 presented tan necrosis on the differentials cvs. Glenlea and Katepwa, chlorosis on line 6B365 and resistance on line 6B662 and differential cv. Salamouni. The presence of necrosis in the differentials Glenlea and Katepwa were expected, since the genotypic characterization of

these four isolates demonstrated the presence of the *ToxA* gene. This gene is responsible for the production of Ptr Tox-A which causes the typical necrotic lesions in susceptible wheat cultivars (Moreno et al. 2012).

Interestingly the presence of extensive chlorosis in line 6B365 suggests the presence of the *ToxC* gene. Studies have shown that the presence of chlorosis in line 6B365 is often associated with the production of Ptr Tox-C (Andrie et al. 2007). However, the lack of molecular markers available for determining the presence or absence the *ToxC* gene was a limiting factor in the present study and restricted the identification of this toxin on our isolates. Furthermore, the four isolates identified as race 1 produced a hypersensitive response in line 6B662 and in the differential Salamouni, which supports the race identification of these isolates as race 1. These results fit in with previous studies describing race 1 as the most prevalent race in the Unites States and Western Canada. (Ali and Francl 2003; Maclean et al. 2017; Moreno et al. 2012; Noriel et al. 2011; Tran et al. 2017). In the United States, studies performed in Arkansas, South Dakota and in the Great Plains have also demonstrated the predominance of race 1 when analyzing the race diversity of *P. tritici-repentis* (Abdullah et al. 2017a; Ali and Francl 2003; Ali et al. 2010). Moreover, in Oklahoma a previous study demonstrated that 15 out of 16 isolates corresponded to race 1, suggesting that race 1 was predominant in the state (Kader 2010). It is possible that race 1 is the most prevalent race due to its ability to produce the two toxins, ToxA and ToxC, which may increase this race's virulence and fitness (Ali and Francl 2003; Kader 2010). Another possible explanation is the absence of selection pressure, due to the monoculture of susceptible cultivars (Abdullah et al. 2017a; Ali and Francl 2003).

Analysis of the race distribution of PTR has indicated that race 5 is rare in North America, while race 6 has only been identified in Algeria (Ciuffetti et al. 2014). Races 7 and 8 have been mainly found in the Middle East, the Caucasus and in Algeria (Ciuffetti et al. 2014), hence there is a low probability to encountering those races in the state of Oklahoma.

Phenotypic evaluation of five PTR isolates also indicate the absence of races 3 and 4. Races 3 and 4 have been found in North America, but with a low frequency (Ciuffetti et al. 2014). In Oklahoma, a previous study showed that from 16 PTR isolates only one isolate was identified as race 4 (Kader 2010). Similarly, in a study of 270 single-spore PTR isolates collected from wheat and non-cereal grasses in the Great Plains, only 5% of isolates obtained from wheat corresponded to race 4 (Ali and Francl 2003). Based on our results and previous literature, the presence of race 4 in Oklahoma is possible; however, its incidence is lower than race 1; therefore, further race characterization of all the isolates collected is suggested to determine if current populations of PTR belong to only race 1 or more races in Oklahoma.

Race classification for one of the isolates analyzed was not possible, since the symptoms produced did not correspond to any of the known races proposed in the scheme by Andrie et al. (2007). This isolate produced necrotic lesions in the differential cv. Glenlea although this isolate lacked the *ToxA* gene according to the genotypic characterization. These results suggest that this isolate could contain another toxin or toxins inducing similar symptoms in Glenlea. Or a technical explanation is that the primer site had mutations that disrupted annealing and the PCR assay for the detection of the *ToxA* gene. Similar results were found in a study in Arkansas where 13 isolates that

induced necrosis on the differential cv. Glenlea did not harbor the *ToxA* gene (Ali et al. 2010). A study in South Dakota also encountered isolates that did not correspond to any known race and were denominated as unknown races (Abdullah et al. 2017a). Thus, it has been proposed that the existence of new, virulent races of PTR might be capable of producing novel toxins that trigger similar symptoms in wheat (Andrie et al. 2007; Ciuffetti et al. 2014; Lepoint et al. 2010; Maclean et al. 2017; Moreno et al. 2012). Regardless, this possibility needs further study, and characterization of the toxins involved is necessary.

Based on these limited results it appears that race 1 is the predominant race in Oklahoma since both phenotypic and genotypic characterizations corroborated on another. However, further analyses are required to determine the presence or absence of the *ToxC* gene or other toxins, for definitive race classifications in Oklahoma populations. Oklahoma PTR populations are genetically diverse (chapter IV), so more complex race population composition could be expected. However, genotypic and phenotypic characterization of a larger number of isolates may be necessary to find isolates from races with low incidence in Oklahoma.

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APPENDICES

Supplementary table 1. *Pyrenophora tritici-repentis* isolates identified morphologically from 13 no-till wheat fields from Oklahoma during 2016.

Isolate Number	Field	Code
1	A	A1-3NC-1
2	A	A1-3NC-2
3	A	A1-5NC
4	A	A2-2NC
5	A	A2-3C-1
6	A	A2-3C-2
7	A	A2-3NC-1
8	A	A2-3NC-2
9	A	A2-3NC-3
10	A	A2-3NC-4
11	A	A2-4NC
12	A	A3-1C
13	A	A4-1C
14	A	A4-2NC-1
15	A	A4-2NC-2
16	A	A4-2NC-3
17	A	A4-2NC-4
18	A	A4-2NC-5
19	A	A4-3NC-1
20	A	A4-3NC-3
21	A	A4-3NC-4
22	A	A4-4NC
23	A	A4-4NC-1
24	A	A4-4NC-3
25	A	A4-4NC-4
26	A	A4-5C
27	A	A4-5NC-1
28	A	A4-5NC-2
29	A	A4-5NC-3
30	A	A5-1NC-1
31	A	A5-1NC-2
32	A	A5-3NC-1

33	A	A5-3NC-2
34	A	A5-3NC-3
35	A	A5-3NC-4
36	A	A5-4NC-1
37	A	A5-4NC-2
38	A	A5-5NC-1
39	A	A5-5NC-2
40	A	A6-3NC-1
41	A	A6-3NC-1
42	A	A6-4NC-1
43	A	A6-4NC-2
44	A	A6-4NC-3
45	A	A6-4NC-4
46	A	A6-4C-1
47	A	A6-4C-2
48	A	A6-5NC-1
49	A	A6-5NC-2
50	A	A7-1NC
51	A	A7-1NC-1
52	A	A7-1NC-3
53	A	A7-1NC-4
54	A	A7-1NC-5
55	A	A7-2NC-1
56	A	A7-2NC-2
57	A	A7-3C
58	A	A7-3NC-1
59	A	A7-3NC-2
60	A	A7-3NC-3
61	A	A7-4NC
62	A	A7-5NC
63	B	B1-1NC-1
64	B	B1-1NC-2
65	B	B1-3C
66	B	B1-3NC
67	B	B1-5NC-1
68	B	B1-5NC-3
69	B	B2-1NC-1
70	B	B2-1NC-2
71	B	B2-1NC-3
72	B	B2-1C-1
73	B	B2-1C-2
74	B	B2-1C-3
75	B	B2-2NC-1

76	B	B2-2NC-2
77	B	B2-2NC-3
78	B	B2-2NC-4
79	B	B2-3NC-1
80	B	B2-3NC-2
81	B	B2-3NC-3
82	B	B2-3NC-4
83	B	B2-3NC-5
84	B	B2-4NC-1
85	B	B2-4NC-2
86	B	B2-4NC-3
87	B	B2-4NC-4
88	B	B2-5NC-1
89	B	B2-5NC-2
90	B	B2-5NC-3
91	B	B2-5NC-4
92	B	B2-5NC-5
93	B	B3-1NC-1
94	B	B3-1NC-2
95	B	B3-1NC-4
96	B	B3-2NC-1
97	B	B3-2NC-2
98	B	B3-2NC-3
99	B	B3-2NC-5
100	B	B3-3NC-1
101	B	B3-3NC-2
102	B	B3-3NC-3
103	B	B3-3NC-4
104	B	B3-4NC
105	B	B3-4NC-1
106	B	B3-4NC-2
107	B	B3-5NC-1
108	B	B3-5NC-2
109	B	B3-5NC-3
110	B	B3-5NC-4
111	B	B3-5NC-5
112	B	B4-1NC
113	B	B4-2NC-1
114	B	B4-2NC-2
115	B	B4-3C
116	B	B4-3NC-1
117	B	B4-3NC-2
118	B	B4-3NC-3

119	B	B4-4NC-2
120	B	B4-4NC-3
121	B	B4-4NC-4
122	B	B4-5NC-1
123	B	B4-5NC-2
124	B	B4-5NC-3
125	B	B5-2NC-1
126	B	B5-2NC-2
127	B	B5-2NC-3
128	B	B5-2NC-4
129	B	B5-4NC-1
130	B	B5-4NC-2
131	B	B5-4NC-3
132	B	B5-5NC-1
133	B	B5-5NC-2
134	B	B5-5NC-3
135	B	B5-5NC-4
136	B	B6-1NC-1
137	B	B6-1NC-2
138	B	B6-1NC-3
139	B	B6-1NC-4
140	B	B6-2NC-1
141	B	B6-2NC-3
142	B	B6-3NC-2
143	B	B6-4N-1
144	B	B6-4N-2
145	B	B6-4NC-1
146	B	B6-4NC-2
147	B	B6-4NC-3
148	B	B6-4NC-4
149	B	B6-4NC-5
150	B	B6-5NC-1
151	B	B6-5NC-2
152	B	B6-5NC-4
153	C	C1-1NC
154	C	C1-1NC-1
155	C	C1-1NC-2
156	C	C1-1NC-3
157	C	C1-1NC-4
158	C	C1-1C-1
159	C	C1-1C-2
160	C	C1-1C-3
161	C	C1-3NC-1

162	C	C1-3NC-2
163	C	C1-3NC-3
164	C	C1-3NC-4
165	C	C1-3C
166	C	C1-4C-1
167	C	C1-4C-2
168	C	C1-4C-3
169	C	C1-4NC-2
170	C	C1-4NC-3
171	C	C1-5NC-1
172	C	C1-5NC-2
173	C	C1-5NC-3
174	C	C1-5NC-4
175	C	C2-1NC-1
176	C	C2-1NC-2
177	C	C2-1NC-3
178	C	C2-1NC-4
179	C	C2-1NC-5
180	C	C2-1NC-6
181	C	C2-2NC-1
182	C	C2-2NC-2
183	C	C2-2NC-3
184	C	C2-2NC-4
185	C	C2-3NC-1
186	C	C2-3NC-6
187	C	C2-4NC
188	C	C2-5NC-1
189	C	C2-5NC-2
190	C	C3-1NC-1
191	C	C3-1NC-2
192	C	C3-1NC-3
193	C	C3-1NC-4
194	C	C3-1C-1
195	C	C3-2C-1
196	C	C3-2C-2
197	C	C3-2NC
198	C	C3-3NC-1
199	C	C3-3NC-2
200	C	C3-4NC-1
201	C	C3-4NC-3
202	C	C3-5C
203	C	C3-5C-1
204	D	D1-1NC-1

205	D	D1-1NC-2
206	D	D1-1NC-3
207	D	D1-2NC-2
208	D	D1-2NC-3
209	D	D1-3NC-1
210	D	D1-3NC-2
211	D	D1-3NC-3
212	D	D1-4NC
213	D	D1-5NC-2
214	D	E1-2NC-1
215	D	E1-2NC-2
216	D	E1-2NC-3
217	D	E1-2NC-4
218	D	E1-3NC-1
219	D	E1-3NC-2
220	D	E1-3NC-3
221	D	E1-4NC
222	D	E1-5NC-1
223	D	E2-3NC-1
224	D	E2-3NC-2
225	D	E2-4NC-1
226	D	E2-4NC-2
227	D	E2-5NC
228	D	F1-2NC-1
229	D	F1-2NC-2
230	D	F1-2NC-3
231	D	F1-3NC-2
232	D	F1-3NC-3
233	D	F1-4NC
234	D	F1-5NC
235	E	G1-1NC
236	E	G1-2NC
237	E	G1-3NC
238	E	G1-4NC
239	F	H1-2NC
240	F	H1-3NC-1
241	F	H1-3NC-2
242	F	H1-3NC-3
243	F	H1-3NC-4
244	F	H1-3NC-5
245	F	H1-4NC-1
246	F	H1-4NC-2
247	F	H1-4NC-3

248	F	H1-4NC-5
249	F	H1-5NC-1
250	F	H1-5NC-2
251	F	H2-1NC-1
252	F	H2-1NC-2
253	F	H2-1NC-3
254	F	H2-2NC-1
255	F	H2-2NC-2
256	F	H2-2NC-3
257	F	H2-3NC
258	F	H2-4NC-2
259	F	H2-5NC-1
260	F	H2-5NC-2
261	F	H2-5NC-3
262	F	H3-1NC
263	F	H3-4NC
264	F	H4-1NC-1
265	F	H4-1NC-2
266	F	H4-1NC-3
267	F	H4-1NC-4
268	F	H4-1NC-5
269	F	H4-2NC-1
270	F	H4-2NC-2
271	F	H4-2NC-3
272	F	H4-2NC-4
273	F	H4-2NC-5
274	F	H4-2NC-6
275	F	H4-3NC-1
276	F	H4-3NC-2
277	F	H4-3NC-3
278	F	H4-3NC-4
279	F	H4-4NC-1
280	F	H4-4NC-2
281	F	H4-4NC-3
282	F	H4-4NC-4
283	F	H4-4NC-5
284	F	H4-5NC-1
285	F	H4-5NC-2
286	F	H4-5NC-3
287	F	H4-5NC-5
288	F	H5-1NC
289	F	H5-2NC-1
290	F	H5-2NC-2

291	F	H5-3NC-1
292	F	H5-3NC-2
293	F	H5-3NC-3
294	F	H5-4NC
295	F	H5-5NC
296	G	I1-1NC
297	G	I1-2NC-1
298	G	I1-3NC
299	G	I2-1NC-1
300	G	I2-2NC-1
301	G	I2-2NC-2
302	G	I2-4NC-1
303	G	I2-4NC-2
304	G	I2-5NC-1
305	G	I3-1NC-1
306	G	I3-1NC-2
307	G	I3-3NC-1
308	G	I3-3NC-2
309	G	I3-3NC-3
310	G	I3-3NC-4
311	G	I3-4NC-1
312	G	I4-1NC-1
313	G	I5-3NC-1
314	G	I5-4NC-1
315	G	I5-4NC-2
316	G	I5-5NC-1
317	G	I5-5NC-2
318	G	I5-5NC-3
319	G	I6-2NC-1
320	G	I6-3NC-1
321	G	I6-3NC-2
322	G	I6-3NC-3
323	G	I6-3NC-4
324	G	I6-4NC-1
325	G	I6-5NC-1
326	G	I6-5NC-2
327	G	I6-5NC-3
328	G	I6-5NC-4
329	G	I7-1NC-1
330	G	I7-1NC-2
331	G	I7-1NC-3
332	G	I7-2NC
333	G	I7-3NC-1

334	G	I7-3NC-2
335	G	I7-4NC-1
336	G	I7-4NC-2
337	G	I7-5NC-1
338	G	I7-5NC-2
339	G	I7-5NC-3
340	H	J1-2C-1
341	H	J1-5NC-1
342	H	J3-1NC-1
343	H	J3-2NC-1
344	H	J3-3NC-1
345	H	J3-3NC-2
346	H	J3-3NC-3
347	H	J3-4NC-1
348	H	J3-4NC-2
349	H	J3-4NC-3
350	H	J3-5NC-1
351	H	J3-5NC-2
352	H	J5-1NC-1
353	H	J5-1NC-2
354	H	J5-2NC-1
355	H	J5-3NC-1
356	H	J5-3NC-2
357	H	J5-3NC-3
358	H	J5-5NC-1
359	H	J5-5NC-2
360	H	J5-5NC-3
361	I	K1-1NC-1
362	I	K1-1NC-2
363	I	K1-1NC-3
364	I	K1-1NC-4
365	I	K1-1NC-5
366	I	K1-2NC-1
367	I	K1-2NC-2
368	I	K1-2NC-3
369	I	K1-3NC-1
370	I	K1-4NC-1
371	I	K1-5NC-1
372	I	K2-2NC-1
373	I	K2-2NC-2
374	I	K2-2NC-3
375	I	K2-4NC-1
376	I	K2-4NC-2

377	I	K2-4NC-3
378	I	K2-4NC-4
379	I	K2-5NC-2
380	I	K3-1NC-1
381	I	K3-2NC-1
382	I	K3-2NC-2
383	I	K3-3NC-1
384	I	K3-4NC-1
385	I	K3-4NC-2
386	I	K4-1NC-1
387	I	K4-1NC-2
388	I	K4-1NC-3
389	I	K4-3NC-1
390	I	K4-4NC-1
391	I	K4-4NC-2
392	I	K4-5NC-1
393	I	K4-5NC-2
394	I	K4-5NC-3
395	I	K5-1NC-1
396	I	K5-1NC-2
397	I	K5-2NC-1
398	I	K5-3NC-1
399	I	K5-3NC-2
400	I	K5-4NC-1
401	J	L1-INC-1
402	J	L1-1NC-2
403	J	L1-2NC-1
404	J	L1-2NC-2
405	J	L1-2NC-3
406	J	L1-2NC-4
407	J	L1-2NC-5
408	J	L1-2NC-6
409	J	L1-3NC-1
410	J	L1-3NC-2
411	J	L1-3NC-3
412	J	L1-3NC-4
413	J	L1-4NC-1
414	J	L1-4NC-3
415	J	L1-4NC-4
416	J	L2-1NC-1
417	J	L2-2NC-1
418	J	L3-1NC-1
419	J	L3-1NC-2

420	J	L3-1NC-3
421	J	L3-1NC-4
422	J	L3-2NC-1
423	J	L3-3NC-1
424	J	L3-3NC-2
425	J	L3-3NC-3
426	J	L3-4NC-1
427	J	L3-4NC-2
428	J	L3-4NC-3
429	J	L3-5NC-1
430	J	L3-5NC-2
431	J	L3-5NC-3
432	J	L3-5NC-4
433	J	L3-5NC-5
434	J	L4-1NC-1
435	J	L4-1NC-2
436	J	L4-1NC-3
437	J	L4-2NC-1
438	J	L4-3NC-1
439	J	L4-3NC-2
440	J	L4-4NC-1
441	J	L4-4NC-3
442	J	L4-4NC-4
443	J	L4-4NC-5
444	J	L4-3NC-1
445	J	L4-5NC-2
446	J	L4-5NC-4
447	J	L4-5NC-5
448	J	L4-5NC-6
449	J	L4-5NC-7
450	J	L5-1NC-1
451	J	L5-1NC-2
452	J	L5-2NC-1
453	J	L5-2NC-2
454	J	L5-2NC-3
455	J	L5-2NC-4
456	J	L5-2NC-5
457	J	L5-3NC-1
458	J	L5-3NC-2
459	J	L5-3NC-3
460	J	L5-3NC-1
461	J	L5-3NC-5
462	J	L5-3NC-6

463	J	L5-4NC-1
464	J	L5-4NC-2
465	J	L5-5NC-2
466	J	L5-5NC-3
467	J	L5-5NC-4
468	J	L6-9NC-1
469	K	M2-2NC-1
470	K	M2-3NC-1
471	K	M2-4NC-1
472	K	M2-4NC-2
473	K	M2-5NC-1
474	K	M2-6NC-1
475	K	M2-8NC-1
476	K	M3-1NC-1
477	K	M3-6NC-1
478	K	M4-1NC-1
479	K	M4-2NC-1
480	K	M4-5NC-1
481	K	M4-6NC-1
482	K	M4-8NC-1
483	K	M4-10NC-1
484	K	M4-10NC-2
485	K	M6-1NC-1
486	L	N1-5NC-1
487	L	N2-4NC-1
488	L	N2-4NC-2
489	L	N3-1NC-1
490	L	N3-2NC-2
491	L	N3-3NC-1
492	L	N3-3NC-2
493	L	N3-4NC-1
494	L	N4-1NC-1
495	L	N4-1NC-2
496	L	N4-3NC-1
497	L	N4-4NC-1
498	L	N4-4NC-2
499	L	N5-1NC-1
500	L	N5-3NC-1
501	L	N5-5NC-1
502	L	N5-5NC-2
503	M	O1-1NC-1
504	M	O1-1NC-2
505	M	O1-3NC-1

506	M	O1-4NC-2
507	M	O1-4NC-3
508	M	O1-5NC-1
509	M	O1-5NC-2
510	M	O1-5NC-5
511	M	O1-5NC-6
512	M	O1-6NC-1
513	M	O1-6NC-3
514	M	O1-8NC-1
515	M	O2-1NC-1
516	M	O2-2NC-1
517	M	O2-2NC-2
518	M	O2-3NC-1
519	M	O2-3NC-2
520	M	O2-4NC-1
521	M	O2-4NC-2
522	M	O2-5NC-1
523	M	O2-7NC-1
524	M	O2-7NC-2
525	M	O2-10NC-2
526	M	O2-10NC-3
527	M	O2-10NC-5
528	M	O3-1NC-1
529	M	O3-1NC-2
530	M	O3-1NC-3
531	M	O3-1NC-4
532	M	O3-1NC-5
533	M	O3-1NC-6
534	M	O3-1NC-7
535	M	O3-2NC-1
536	M	O3-2NC-2
537	M	O3-2NC-3
538	M	O3-3NC-1
539	M	O3-3NC-3
540	M	O3-3NC-4
541	M	O3-4NC-1
542	M	O3-5NC-1
543	M	O3-5NC-2
544	M	O3-5NC-3
545	M	O3-5NC-4
546	M	O3-6NC-1
547	M	O3-6NC-2
548	M	O3-6NC-3

549	M	O3-7NC-1
550	M	O3-7NC-2
551	M	O3-7NC-3
552	M	O3-7NC-4
553	M	O3-7NC-5
554	M	O3-7NC-6
555	M	03-7NC-7
556	M	03-7NC-8
557	M	O3-8NC-1
558	M	O3-9NC-1
559	M	O3-9NC-2
560	M	O3-9NC-4
561	M	O3-10NC-1
562	M	O3-10NC-2
563	M	O3-10NC-3
564	M	O3-10NC-4
565	M	O3-10NC-5

Supplementary table 2. Pycnidial isolates identified morphologically from 13 no-till wheat fields from Oklahoma during 2016

Isolate Number	Field	Code
1	A	A1-1NC-A
2	A	A1-1NC-B
3	A	A1-1N-A
4	A	A1-1N-B
5	A	A1-1N-C
6	A	A1-2NC-A
7	A	A1-2NC-B
8	A	A1-2NC-C
9	A	A1-3NC-A
10	A	A1-3NC-B
11	A	A1-4C-A
12	A	A1-4C-B
13	A	A1-4NC-A
14	A	A1-4N-A
15	A	A1-5N-A
16	A	A1-5NC-A
17	A	A2-1NC
18	A	A2-2NC-A
19	A	A2-3C-A
20	A	A2-3C-B

21	A	A2-3N-B
22	A	A2-3N-C
23	A	A2-4NC-A
24	A	A2-4NC-B
25	A	A2-4NC-C
26	A	A2-4NC-D
27	A	A2-4C-A
28	A	A2-4C-B
29	A	A2-4C-C
30	A	A2-5N-A
31	A	A2-5NC-A
32	A	A3-1C-A
33	A	A3-1C-B
34	A	A3-2NC-B
35	A	A3-2NC-D
36	A	A3-2NC-E
37	A	A3-3NC
38	A	A3-3NC-A
39	A	A3-3NC-B
40	A	A3-3NC-C
41	A	A3-3NC-D
42	A	A3-3NC-E
43	A	A3-3N-A
44	A	A3-3N-C
45	A	A3-3N-D
46	A	A3-3N-E
47	A	A3-4NC-A
48	A	A3-4NC-B
49	A	A3-4N-A
50	A	A3-5NC-B
51	A	A3-5N-A
52	A	A3-5N-B
53	A	A3-5N-C
54	A	A3-5N-D
55	A	A3-5N-E
56	A	A5-3NC
57	A	A6-4NC-A
58	A	A7-1NC-A
59	B	B1-1C
60	B	B1-3C
61	B	B1-3NC-B
62	B	B1-5C
63	B	B1-5NC-A

64	B	B1-5NC-B
65	B	B1-5NC-C
66	B	B2-1NC-A
67	B	B3-1C
68	B	B5-1NC
69	B	B6-1NC
70	C	C1-2NC
71	C	C1-2C-B
72	C	C1-3C
73	C	C1-3NC-B
74	C	C3-3NC-A
75	D	D1-3NC-A
76	D	D1-4NC
77	E	G1-1NC-A
78	E	G1-1NC-B
79	E	G1-2NC
80	E	G1-3NC-B
81	E	G1-4NC-A
82	E	G1-4NC-B
83	E	G1-5NC-A
84	E	G1-5NC-B
85	F	H1-1NC-A
86	F	H1-1NC-B
87	F	H1-1NC-C
88	F	H1-1NC-D
89	F	H1-1NC-E
90	F	H1-2NC-B
91	F	H1-3NC
92	F	H1-5NC-A
93	F	H1-5NC-B
94	F	H2-2NC-A
95	F	H2-2NC-B
96	F	H2-3NC-A
97	F	H2-3NC-B
98	F	H2-4NC-A
99	F	H2-4NC-B
100	F	H2-4NC-C
101	F	H2-4NC-D
102	F	H2-5NC-A
103	F	H2-5NC-B
104	F	H3-1NC-B
105	F	H3-2NC-A
106	F	H3-2NC-B

107	F	H3-2NC-C
108	F	H3-2NC-D
109	F	H3-5NC-B
110	F	H3-5NC-C
111	F	H4-3NC
112	F	H5-1NC-A
113	F	H5-1NC-B
114	F	H5-1NC-C
115	F	H5-1NC-D
116	F	H5-2NC-A
117	F	H5-2NC-B
118	F	H5-3NC-A
119	F	H5-4NC-A
120	F	H5-4NC-B
121	F	H5-5NC-A
122	F	H5-5NC-B
123	F	H5-5NC-C
124	G	I1-2NC-A
125	G	I1-2NC-B
126	G	I1-3NC-A
127	G	I1-3NC-B
128	G	I1-3NC-C
129	G	I1-5NC-B
130	G	I1-5NC-C
131	G	I1-5NC-D
132	G	I2-1NC-A
133	G	I2-1NC-B
134	G	I2-3NC-B
135	G	I2-4NC-A
136	G	I2-4NC-B
137	G	I2-5NC-A
138	G	I3-1NC-A
139	G	I3-1NC-B
140	G	I3-1NC-C
141	G	I3-3NC-A
142	G	I3-4NC-A
143	G	I3-5NC-A
144	G	I3-5NC-B
145	G	I3-5NC-C
146	G	I4-1NC-A
147	G	I4-1NC-B
148	G	I4-1NC-C
149	G	I4-2NC-A

150	G	I4-4NC-A
151	G	I4-4NC-B
152	G	I4-5NC-A
153	G	I4-5NC-B
154	G	I4-5NC-C
155	G	I4-5NC-D
156	G	I5-1NC-B
157	G	I5-1NC-C
158	G	I5-3NC-A
159	G	I5-4NC-A
160	G	I5-4NC-B
161	G	I5-5NC-A
162	G	I6-1NC-A
163	G	I6-1NC-B
164	G	I6-1NC-C
165	G	I6-1NC-D
166	G	I6-2NC-A
167	G	I6-4NC-A
168	G	I6-4NC-B
169	G	I7-2NC
170	G	I7-4NC-A
171	G	I7-4NC-B
172	G	I7-5NC-A
173	G	I7-5NC-B
174	H	J3-2NC-A
175	H	J3-3NC-A
176	H	J3-3NC-B
177	H	J3-5N-A
178	H	J5-2NC-A
179	H	J5-2NC-B
180	J	L1-4NC-A
181	J	L1-5NC-A
182	J	L1-5NC-B
183	J	L4-1NC-A
184	J	L4-1NC-B
185	J	L4-3NC-A
186	J	L4-4NC-A
187	K	M3-6NC-A
188	L	N5-1NC-A
189	L	N5-2NC-A
190	L	N5-4NC-A

Supplementary table 3. PCR-positive single-spore PTR isolates obtained from 13 no-till wheat fields from Oklahoma during 2016 and 2017.

Isolate Number	Field	Code	Year of collection
1	A	A1-3NC-1	2016
2	A	A1-5NC	2016
3	A	A2-2NC	2016
4	A	A2-3NC-1	2016
5	A	A2-3NC-3	2016
6	A	A2-3NC-4	2016
7	A	A2-3C-1	2016
8	A	A2-4NC	2016
9	A	A4-2NC-1	2016
10	A	A4-2NC-2	2016
11	A	A4-2NC-3	2016
12	A	A4-2NC-4	2016
13	A	A4-2NC-5	2016
14	A	A4-3NC-1	2016
15	A	A4-4NC	2016
16	A	A4-4NC-3	2016
17	A	A4-5NC-1	2016
18	A	A4-5NC-2	2016
19	A	A5-1NC-2	2016
20	A	A5-3NC-1	2016
21	A	A5-3NC-2	2016
22	A	A5-4NC-2	2016
23	A	A5-5NC-1	2016
24	A	A5-5NC-2	2016
25	A	A6-3NC-1	2016
26	A	A6-3NC-2	2016
27	A	A6-4C-1	2016
28	A	A6-4C-2	2016
29	A	A6-4NC-1	2016
30	A	A6-4NC-3	2016
31	A	A6-4NC-4	2016
32	A	A6-5NC-1	2016
33	A	A6-5NC-2	2016
34	A	A7-1NC-3	2016
35	A	A7-1NC-4	2016
36	A	A7-2NC-1	2016
37	A	A7-2NC-2	2016
38	A	A7-3C	2016
39	A	A7-3NC-1	2016

40	A	A7-3NC-2	2016
41	A	A7-3NC-3	2016
42	A	A7-4NC	2016
43	A	A7-5NC	2016
44	A	A2-1NC-1	2017
45	A	A2-1NC-2	2017
46	A	A2-2NC-2	2017
47	A	A2-3NC-3	2017
48	A	A2-3NC-4	2017
49	A	A2-4NC-1	2017
50	A	A3-2NC-2	2017
51	A	A3-3NC-1	2017
52	A	A4-1NC-1	2017
53	A	A4-3NC-1	2017
54	B	B1-1NC-2	2016
55	B	B1-2NC-1	2016
56	B	B1-3NC	2016
57	B	B1-5C	2016
58	B	B1-5NC-3	2016
59	B	B2-1C-3	2016
60	B	B2-1NC-1	2016
61	B	B2-1NC-2	2016
62	B	B2-1NC-3	2016
63	B	B2-2NC-2	2016
64	B	B2-2NC-3	2016
65	B	B2-2NC-4	2016
66	B	B2-3NC-1	2016
67	B	B2-3NC-2	2016
68	B	B2-3NC-5	2016
69	B	B2-4NC-1	2016
70	B	B2-4NC-2	2016
71	B	B2-4NC-3	2016
72	B	B2-4NC-4	2016
73	B	B2-5NC-2	2016
74	B	B2-5NC-4	2016
75	B	B3-1NC-1	2016
76	B	B3-1NC-2	2016
77	B	B3-2NC-1	2016
78	B	B3-2NC-2	2016
79	B	B3-2NC 3	2016
80	B	B3-2NC-5	2016
81	B	B3-3NC-1	2016
82	B	B3-3NC-2	2016

83	B	B3-3NC-3	2016
84	B	B3-3NC-4	2016
85	B	B3-4NC-1	2016
86	B	B3-4NC-2	2016
87	B	B3-5NC-1	2016
88	B	B3-5NC-2	2016
89	B	B3-5NC-3	2016
90	B	B3-5NC-4	2016
91	B	B3-5NC-5	2016
92	B	B4-2NC-1	2016
93	B	B4-3C	2016
94	B	B4-3NC-1	2016
95	B	B4-3NC-2	2016
96	B	B4-4NC-3	2016
97	B	B4-5NC-1	2016
98	B	B5-2NC-1	2016
99	B	B5-2NC-3	2016
100	B	B5-4NC-1	2016
101	B	B5-4NC-3	2016
102	B	B5-5NC-1	2016
103	B	B5-5NC-2	2016
104	B	B6-1C-3	2016
105	B	B6-1NC-1	2016
106	B	B6-1NC 2	2016
107	B	B6-1NC-3	2016
108	B	B6-2NC-3	2016
109	B	B6-3NC-2	2016
110	B	B6-4N-2	2016
111	B	B6-4NC-1	2016
112	B	B6-4NC-2	2016
113	B	B6-4NC-3	2016
114	B	B6-4NC-5	2016
115	B	B6-5NC-1	2016
116	B	B6-5NC-2	2016
117	C	C1-1NC-1	2016
118	C	C1-1NC-2	2016
119	C	C1-3NC 1	2016
120	C	C1-3NC 2	2016
121	C	C1-3NC-3	2016
122	C	C1-3NC-4	2016
123	C	C1-4C-1	2016
124	C	C1-5NC-2	2016
125	C	C1-5NC-3	2016

126	C	C1-5NC 4	2016
127	C	C2-1NC-3	2016
128	C	C2-1NC-4	2016
129	C	C2-1NC-5	2016
130	C	C2-1NC 6	2016
131	C	C2-2NC-3	2016
132	C	C2-5NC-2	2016
133	C	C3-1NC-1	2016
134	C	C3-1NC-2	2016
135	C	C3-1NC-4	2016
136	C	C3-3NC-1	2016
137	C	C3-4NC-1	2016
138	E	E1-5NC-1	2016
139	F	H1-2NC	2016
140	F	H1-3NC-1	2016
141	F	H1-3NC-2	2016
142	F	H1-3NC-3	2016
143	F	H1-3NC-4	2016
144	F	H1-3NC-5	2016
145	F	H1-4NC-1	2016
146	F	H1-4NC-2	2016
147	F	H1-5NC-2	2016
148	F	H2-1NC-1	2016
149	F	H2-1NC-2	2016
150	F	H2-1NC-3	2016
151	F	H2-2NC-1	2016
152	F	H2-2NC-3	2016
153	F	H2-5NC-1	2016
154	F	H4-1NC-1	2016
155	F	H4-1NC-3	2016
156	F	H4-1NC-4	2016
157	F	H4-1NC-5	2016
158	F	H4-2NC-1	2016
159	F	H4-2NC-2	2016
160	F	H4-2NC-3	2016
161	F	H4-2NC-4	2016
162	F	H4-2NC-6	2016
163	F	H4-3NC-2	2016
164	F	H4-3NC-3	2016
165	F	H4-3NC-4	2016
166	F	H4-4NC-1	2016
167	F	H4-4NC-2	2016
168	F	H4-4NC-3	2016

169	F	H4-4NC-4	2016
170	F	H4-4NC-5	2016
171	F	H4-5NC-1	2016
172	F	H4-5NC-5	2016
173	F	H5-1NC	2016
174	F	H5-2NC-2	2016
175	F	H5-3NC-2	2016
176	F	H5-3NC-3	2016
177	F	H5-5NC	2016
178	F	H1-1NC-1	2017
179	F	H1-3NC-1	2017
180	F	H1-3NC-3	2017
181	F	H3-1NC-1	2017
182	F	H3-4NC-3	2017
183	F	H3-5NC-1	2017
184	F	H3-5NC-2	2017
185	F	H3-5NC-3	2017
186	F	H4-4NC-2	2017
187	F	H4-4NC-3	2017
188	F	H5-3NC-1	2017
189	F	H5-4NC-1	2017
190	F	H5-4NC-2	2017
191	G	I2-2NC-1	2016
192	G	I2-4NC-1	2016
193	G	I2-4NC-2	2016
194	G	I2-5NC-1	2016
195	G	I3-1NC-2	2016
196	G	I3-3NC-1	2016
197	G	I3-3NC-2	2016
198	G	I3-4NC-1	2016
199	G	I4-1NC-1	2016
200	G	I5-4NC-2	2016
201	G	I6-2NC-1	2016
202	G	I6-3NC-1	2016
203	G	I6-4NC-1	2016
204	G	I6-5NC 2	2016
205	G	I6-5NC-3	2016
206	G	I6-5NC-4	2016
207	G	I7-5NC-2	2016
208	G	I7-5NC-3	2016
209	H	J3-1NC-1	2016
210	H	J3-3NC-2	2016
211	H	J3-3NC-3	2016

212	H	J3-4NC-2	2016
213	H	J3-4NC-3	2016
214	H	J5-1NC-1	2016
215	H	J5-1NC-2	2016
216	H	J5-2NC-1	2016
217	H	J5-3NC-1	2016
218	H	J5-3NC-2	2016
219	H	J5-5NC-3	2016
220	I	K1-1NC-1	2016
221	I	K1-1NC-3	2016
222	I	K1-1NC-5	2016
223	I	K1-2NC-1	2016
224	I	K1-2NC-2	2016
225	I	K1-3NC-1	2016
226	I	K1-4NC-1	2016
227	I	K1-5NC-1	2016
228	I	K2-2NC-2	2016
229	I	K2-2NC-3	2016
230	I	K2-4NC-1	2016
231	I	K2-4NC-2	2016
232	I	K2-4NC-3	2016
233	I	K2-4NC-4	2016
234	I	K2-5NC-2	2016
235	I	K3-1NC-1	2016
236	I	K3-2NC-1	2016
237	I	K3-2NC-2	2016
238	I	K3-3NC-1	2016
239	I	K3-4NC-1	2016
240	I	K3-4NC-2	2016
241	I	K4-1NC-1	2016
242	I	K4-1NC-2	2016
243	I	K4-3NC-1	2016
244	I	K4-4NC-1	2016
245	I	K4-4NC-2	2016
246	I	K4-5NC-1	2016
247	I	K4-5NC-3	2016
248	I	K5-1NC-1	2016
249	I	K5-1NC-2	2016
250	I	K5-2NC-1	2016
251	I	K5-3NC-1	2016
252	I	K5-3NC-2	2016
253	I	K5-4NC-1	2016
254	I	K1-1NC-1	2017

255	I	K1-1NC-3	2017
256	I	K1-2NC-2	2017
257	I	K1-2NC-3	2017
258	I	K1-2NC-4	2017
259	I	K1-2NC-5	2017
260	I	K1-3NC-3	2017
261	I	K1-4NC-1	2017
262	I	K1-4NC-3	2017
263	I	K1-4NC-4	2017
264	I	K2-1NC-4	2017
265	I	K2-2NC-3	2017
266	I	K2-2NC-4	2017
267	I	K2-3NC-2	2017
268	I	K2-3NC-3	2017
269	I	K2-3NC-4	2017
270	I	K2-4NC-1	2017
271	I	K2-4NC-2	2017
272	I	K2-4NC-4	2017
273	I	K3-1NC-3	2017
274	I	K3-2NC-1	2017
275	I	K3-3NC-2	2017
276	I	K3-3NC-3	2017
277	I	K3-4NC-1	2017
278	I	K3-4NC-4	2017
279	I	K4-1NC-1	2017
280	I	K4-1NC-5	2017
281	I	K4-2NC-4	2017
282	I	K4-2NC-5	2017
283	I	K4-3NC-1	2017
284	I	K4-3NC-5	2017
285	I	K4-4NC-1	2017
286	J	L1-2NC-1	2016
287	J	L1-2NC-2	2016
288	J	L1-2NC-5	2016
289	J	L1-3NC-1	2016
290	J	L1-3NC-4	2016
291	J	L1-4NC-4	2016
292	J	L2-1NC-1	2016
293	J	L3-1NC-2	2016
294	J	L3-1NC-4	2016
295	J	L3-3NC-3	2016
296	J	L3-4NC-1	2016
297	J	L3-4NC-2	2016

298	J	L3-4NC-3	2016
299	J	L3-5NC-1	2016
300	J	L3-5NC-2	2016
301	J	L3-5NC-3	2016
302	J	L3-5NC-4	2016
303	J	L3-5NC-5	2016
304	J	L4-1NC-1	2016
305	J	L4-1NC-2	2016
306	J	L4-1NC-3	2016
307	J	L4-2NC-1	2016
308	J	L4-3NC-2	2016
309	J	L4-4NC-1	2016
310	J	L4-4NC-3	2016
311	J	L4-4NC-4	2016
312	J	L4-4NC-5	2016
313	J	L4-5NC-2	2016
314	J	L4-5NC-5	2016
315	J	L5-1NC-1	2016
316	J	L5-1NC-2	2016
317	J	L5-2NC-2	2016
318	J	L5-3NC-1	2016
319	J	L5-3NC-2	2016
320	J	L5-3NC-3	2016
321	J	L5-3NC-4	2016
322	J	L5-3NC-5	2016
323	J	L5-3NC-6	2016
324	J	L5-4NC-2	2016
325	J	L5-5NC-2	2016
326	J	L5-5NC-3	2016
327	J	L1-1NC-2	2017
328	J	L1-3NC-2	2017
329	J	L1-3NC-3	2017
330	J	L1-5NC-2	2017
331	J	L1-5NC-4	2017
332	J	L2-1NC-1	2017
333	J	L2-3NC-1	2017
334	J	L2-3NC-3	2017
335	J	L2-4NC-1	2017
336	J	L3-2NC-2	2017
337	J	L3-2NC-3	2017
338	J	L3-3NC-2	2017
339	J	L3-4NC-1	2017
340	J	L3-5NC-2	2017

341	J	L4-1NC-1	2017
342	J	L4-1NC-3	2017
343	J	L4-2NC-3	2017
344	J	L4-2NC-5	2017
345	J	L4-3NC-1	2017
346	J	L4-4NC-2	2017
347	J	L5-4NC-1	2017
348	J	L5-5NC-1	2017
349	J	L5-5NC-2	2017
350	K	M2-3NC-1	2016
351	K	M2-4NC-1	2016
352	K	M2-8NC-1	2016
353	K	M3-6NC-1	2016
354	K	M4-1NC-1	2016
355	K	M4-2NC-1	2016
356	K	M4-6NC-1	2016
357	K	M4-8NC-1	2016
358	K	M4-10NC-1	2016
359	L	N1-5NC-1	2016
360	L	N3-1NC-1	2016
361	L	N3-2NC-2	2016
362	L	N3-3NC-2	2016
363	L	N3-4NC-1	2016
364	L	N4-1NC-1	2016
365	L	N4-1NC-2	2016
366	L	N4-3NC-1	2016
367	L	N4-4NC-2	2016
368	L	N5-1NC-1	2016
369	L	N5-5NC-1	2016
370	L	N5-5NC-2	2016
371	M	O1-4NC-2	2016
372	M	O1-5NC-6	2016
373	M	O2-2NC-2	2016
374	M	O2-7NC-2	2016
375	M	O3-1NC-1	2016
376	M	O3-1NC-2	2016
377	M	O3-1NC-3	2016
378	M	O3-1NC-6	2016
379	M	O3-3NC-1	2016
380	M	O3-3NC-3	2016
381	M	O3-4NC-1	2016
382	M	O3-5NC-3	2016
383	M	O3-6NC-1	2016

384	M	O3-6NC-3	2016
385	M	O3-7NC-2	2016
386	M	O3-7NC-7	2016
387	M	O3-8NC-1	2016
388	M	03-9NC-1	2016
389	M	O3-10NC-4	2016

Supplementary table 4. PCR-negative single-spore PTR isolates obtained from 13 no-till wheat fields from Oklahoma during 2016 and 2017.

Isolate Number	Field	Code	Year of collection
1	A	A1-3NC-2	2016
2	A	A3-1C	2016
3	A	A4-1C	2016
4	A	A4-3NC-3	2016
5	A	A4-5C	2016
6	A	A5-1NC-1	2016
7	A	A5-3NC-3	2016
8	A	A6-4NC-3	2016
9	A	A3-2NC-1	2017
10	A	A4-1NC-2	2017
11	B	B2-2NC-1	2016
12	B	B2-5NC-3	2016
13	B	B3-4NC	2016
14	B	B4-1NC	2016
15	B	B4-2NC-2	2016
16	B	B4-4NC-2	2016
17	B	B4-4NC-4	2016
18	B	B4-5NC 2	2016
19	B	B5-2NC-2	2016
20	B	B5-2NC-4	2016
21	B	B5-4NC-2	2016
22	B	B5-5NC-3	2016
23	B	B6-2NC	2016
24	B	B6-4NC-4	2016
25	B	B6-5NC-4	2016
26	C	C1-1C-2	2016
27	C	C1-1C-3	2016
28	C	C1-3C	2016
29	C	C1-4C-2	2016
30	C	C1-4C-3	2016
31	C	C1-1NC	2016

32	C	C1-1NC-2	2016
33	C	C1-1NC-4	2016
34	C	C1-4NC-2	2016
35	C	C1-5NC-1	2016
36	C	C2-1NC-1	2016
37	C	C2-2NC-1	2016
38	C	C2-2NC-2	2016
39	C	C2-2NC-4	2016
40	C	C2-3NC-1	2016
41	C	C2-3NC-6	2016
42	C	C2-5NC-1	2016
43	C	C3-1C-1	2016
44	C	C3-1NC-3	2016
45	C	C3-2NC	2016
46	C	C3-2C-1	2016
47	C	C3-2C 2	2016
48	C	C3-3NC-2	2016
49	C	C3-4NC-3	2016
50	C	C3-5C	2016
51	D	D1-1NC-1	2016
52	D	D1-1NC-2	2016
53	D	D1-1NC-3	2016
54	D	D1-2NC-2	2016
55	D	D1-2NC-3	2016
56	D	D1-3NC-1	2016
57	D	D1-3NC-2	2016
58	D	D1-3NC-3	2016
59	D	D1-4NC	2016
60	D	D1-5NC-2	2016
61	D	E1-2NC-1	2016
62	D	E1-2NC-2	2016
63	D	E1-2NC-3	2016
64	D	E1-2NC-4	2016
65	D	E1-3NC-1	2016
66	D	E1-3NC-2	2016
67	D	E1-3NC-3	2016
68	D	E1-4NC	2016
69	D	E2-3NC-1	2016
70	D	E2-4NC-1	2016
71	D	E2-4NC-2	2016
72	D	E2-5NC	2016
73	D	F1-2NC-1	2016
74	D	F1-2NC-2	2016

75	D	F1-2NC-3	2016
76	D	F1-3NC-2	2016
77	D	F1-3NC-3	2016
78	D	F1-4NC	2016
79	D	F1-5NC	2016
80	E	G1-1NC	2016
81	E	G1-2NC	2016
82	E	G1-3NC	2016
83	E	G1-4NC	2016
84	F	H1-4NC-5	2016
85	F	H2-1NC-3	2016
86	F	H2-3NC	2016
87	F	H2-5NC-3	2016
88	F	H3-1NC	2016
89	F	H4-1NC-2	2016
90	F	H4-3NC-1	2016
91	F	H4-5NC-2	2016
92	F	H4-5NC 3	2016
93	F	H5-3NC-1	2016
94	F	H5-4NC	2016
95	F	H1-4NC-2	2017
96	F	H2-1NC-1	2017
97	F	H2-5NC-1	2017
98	F	H3-2NC-1	2017
99	F	H3-4NC-1	2017
100	F	H3-4NC-2	2017
101	F	H4-1NC-2	2017
102	F	H4-1NC-3	2017
103	F	H4-3NC-2	2017
104	F	H4-3NC-3	2017
105	F	H5-1NC-2	2017
106	F	H5-3NC-2	2017
107	F	H5-5NC-1	2017
108	G	I1-3NC	2016
109	G	I2-INC-1	2016
110	G	I3-1NC-1	2016
111	G	I3-3NC 3	2016
112	G	I3-3NC-4	2016
113	G	I5-4NC-1	2016
114	G	I5-5NC-1	2016
115	G	I5-5NC-3	2016
116	G	I6-3NC-2	2016
117	G	I6-3NC-4	2016

118	G	I6-5NC 1	2016
119	G	I7-1NC-1	2016
120	G	I7-1NC-2	2016
121	G	I7-1NC-3	2016
122	G	I7-2NC	2016
123	G	I7-3NC-2	2016
124	G	I7-4NC-1	2016
125	G	I7-5NC-1	2016
126	H	J1-2C-1	2016
127	H	J3-4NC-1	2016
128	H	J3-5NC-1	2016
129	H	J3-5NC-2	2016
130	H	J5-3NC-3	2016
131	H	J5-5NC 1	2016
132	I	K1-1NC-2	2016
133	I	K1-1NC-4	2016
134	I	K1-2NC-3	2016
135	I	K2-2NC-1	2016
136	I	K4-1NC-3	2016
137	I	K4-5NC-2	2016
138	I	K1-1NC-2	2017
139	I	K1-1NC-5	2017
140	I	K1-3NC-2	2017
141	I	K1-4NC-2	2017
142	I	K2-1NC-3	2017
143	I	K2-1NC-5	2017
144	I	K2-2NC-1	2017
145	I	K2-2NC-2	2017
146	I	K2-4NC-3	2017
147	I	K3-1NC-2	2017
148	I	K3-2NC-2	2017
149	I	K3-2NC-3	2017
150	I	K3-2NC-4	2017
151	I	K3-3NC-1	2017
152	I	K3-4NC-3	2017
153	I	K4-1NC-3	2017
154	I	K4-2NC-3	2017
155	I	K4-3NC-3	2017
156	I	K4-4NC-3	2017
157	J	L1-2NC-6	2016
158	J	L1-3NC-2	2016
159	J	L1-3NC-3	2016
160	J	L1-4NC-1	2016

161	J	L5-4NC-1	2016
162	J	L6-9NC-1	2016
163	J	L1-1NC-1	2017
164	J	L1-1NC-3	2017
165	J	L1-2NC-4	2017
166	J	L1-3NC-1	2017
167	J	L1-4NC-2	2017
168	J	L1-4NC-3	2017
169	J	L1-5NC-1	2017
170	J	L1-5NC-3	2017
171	J	L1-5NC-5	2017
172	J	L2-1NC-2	2017
173	J	L2-2NC-2	2017
174	J	L2-5NC-1	2017
175	J	L2-5NC-2	2017
176	J	L3-2NC-1	2017
177	J	L3-2NC-4	2017
178	J	L3-3NC-1	2017
179	J	L3-3NC-3	2017
180	J	L4-1NC-4	2017
181	J	L4-2NC-1	2017
182	J	L4-3NC-2	2017
183	J	L4-4NC-1	2017
184	J	L4-5NC-1	2017
185	J	L5-1NC-1	2017
186	J	L5-2NC-1	2017
187	J	L5-2NC-2	2017
188	J	L5-3NC-1	2017
189	J	L5-5NC-3	2017
190	K	M2-2NC-1	2016
191	K	M2-4NC-2	2016
192	K	M2-6NC-1	2016
193	K	M3-1NC-1	2016
194	K	M4-5NC-1	2016
195	K	M6-1NC-1	2016
196	L	N2-4NC-1	2016
197	L	N3-3NC-1	2016
198	L	N4-4NC-1	2016
199	L	N5-3NC-1	2016
200	M	O1-1NC-1	2016
201	M	O1-1NC-2	2016
202	M	O1-3NC-1	2016
203	M	O1-5NC-5	2016

204	M	O1-6NC-1	2016
205	M	O2-2NC-1	2016
206	M	O2-4NC-1	2016
207	M	O2-4NC-2	2016
208	M	O2-5NC-1	2016
209	M	O2-7NC-1	2016
210	M	O2-10NC-2	2016
211	M	O2-10NC-3	2016
212	M	O3-1NC-3	2016
213	M	O3-2NC-1	2016
214	M	O3-2NC-2	2016
215	M	O3-2NC-3	2016
216	M	O3-3NC-4	2016
217	M	O3-5NC-1	2016
218	M	O3-5NC-4	2016
219	M	O3-7NC-1	2016
220	M	O3-7NC-3	2016
221	M	O3-7NC-4	2016
222	M	O3-7NC-5	2016
223	M	O3-9NC-2	2016
224	M	O3-9NC-4	2016
225	M	O3-10NC-2	2016
226	M	O3-10NC-5	2016
227	M	O4-1NC-1	2016

Supplementary table 5. PTR isolates used for host selective toxin production genotyping from 13 no-till wheat fields from Oklahoma during 2016.

Isolate Number	**Field**	**Code**	***Tox A***	***Tox B***
1	A	A1-3NC-1	+	-
2	A	A1-5NC	+	-
3	A	A2-2NC	+	-
4	A	A2-3NC-1	+	-
5	A	A2-3NC-3	+	-
6	A	A2-3NC-4	+	-
7	A	A2-3C-1	+	-
8	A	A2-4NC	+	-
9	A	A4-2NC-1	+	-
10	A	A4-2NC-2	+	-
11	A	A4-2NC-3	+	-
12	A	A4-2NC-4	+	-
13	A	A4-2NC-5	+	-

14	A	A4-3NC-1	+	-
15	A	A4-4NC	+	-
16	A	A4-4NC-3	+	-
17	A	A4-5NC-1	+	-
18	A	A4-5NC-2	-	-
19	A	A5-1NC-2	+	-
20	A	A5-3NC-1	+	-
21	A	A5-3NC-2	+	-
22	A	A5-4NC-2	+	-
23	A	A5-5NC-1	+	-
24	A	A5-5NC-2	+	-
25	A	A6-3NC-1	+	-
26	A	A6-3NC-2	+	-
27	A	A6-4C-1	+	-
28	A	A6-4C-2	+	-
29	A	A6-4NC-1	+	-
30	A	A6-4NC-3	+	-
31	A	A6-4NC-4	+	-
32	A	A6-5NC-1	+	-
33	A	A6-5NC-2	+	-
34	A	A7-1NC-3	+	-
35	A	A7-1NC-4	+	-
36	A	A7-2NC-1	+	-
37	A	A7-2NC-2	+	-
38	A	A7-3C	+	-
39	A	A7-3NC-1	+	-
40	A	A7-3NC-2	+	-
41	A	A7-3NC-3	+	-
42	A	A7-4NC	+	-
43	A	A7-5NC	+	-
44	B	B1-1NC-2	+	-
45	B	B1-2NC-1	+	-
46	B	B1-3NC	+	-
47	B	B1-5C	+	-
48	B	B1-5NC-3	+	-
49	B	B2-1C-3	+	-
50	B	B2-1NC-1	+	-
51	B	B2-1NC-2	+	-
52	B	B2-1NC-3	+	-
53	B	B2-2NC-2	+	-
54	B	B2-2NC-3	+	-
55	B	B2-2NC-4	+	-
56	B	B2-3NC-1	+	-

57	B	B2-3NC-2	+	-
58	B	B2-3NC-5	+	-
59	B	B2-4NC-1	+	-
60	B	B2-4NC-2	+	-
61	B	B2-4NC-3	+	-
62	B	B2-4NC-4	+	-
63	B	B2-5NC-2	+	-
64	B	B2-5NC-4	+	-
65	B	B3-1NC-1	+	-
66	B	B3-1NC-2	+	-
67	B	B3-2NC-1	+	-
68	B	B3-2NC-2	+	-
69	B	B3-2NC 3	+	-
70	B	B3-2NC-5	+	-
71	B	B3-3NC-1	+	-
72	B	B3-3NC-2	+	-
73	B	B3-3NC-3	+	-
74	B	B3-3NC-4	+	-
75	B	B3-4NC-1	+	-
76	B	B3-4NC-2	+	-
77	B	B3-5NC-1	-	-
78	B	B3-5NC-2	+	-
79	B	B3-5NC-3	+	-
80	B	B3-5NC-4	+	-
81	B	B3-5NC-5	+	-
82	B	B4-2NC-1	+	-
83	B	B4-3C	+	-
84	B	B4-3NC-1	-	-
85	B	B4-3NC-2	+	-
86	B	B4-4NC-3	+	-
87	B	B4-5NC-1	+	-
88	B	B5-2NC-1	+	-
89	B	B5-2NC-3	+	-
90	B	B5-4NC-1	+	-
91	B	B5-4NC-3	+	-
92	B	B5-5NC-1	+	-
93	B	B5-5NC-2	+	-
94	B	B6-1C-3	+	-
95	B	B6-1NC-1	+	-
96	B	B6-1NC 2	+	-
97	B	B6-1NC-3	+	-
98	B	B6-2NC-3	+	-
99	B	B6-3NC-2	+	-

100	B	B6-4N-2	+	-
101	B	B6-4NC-1	+	-
102	B	B6-4NC-2	+	-
103	B	B6-4NC-3	+	-
104	B	B6-4NC-5	+	-
105	B	B6-5NC-1	+	-
106	B	B6-5NC-2	+	-
107	C	C1-1NC-1	+	-
108	C	C1-1NC-2	+	-
109	C	C1-3NC 1	+	-
110	C	C1-3NC 2	+	-
111	C	C1-3NC-3	+	-
112	C	C1-3NC-4	+	-
113	C	C1-4C-1	+	-
114	C	C1-5NC-2	+	-
115	C	C1-5NC-3	+	-
116	C	C1-5NC 4	+	-
117	C	C2-1NC-3	+	-
118	C	C2-1NC-4	+	-
119	C	C2-1NC-5	+	-
120	C	C2-1NC 6	-	-
121	C	C2-2NC-3	+	-
122	C	C2-5NC-2	+	-
123	C	C3-1NC-1	+	-
124	C	C3-1NC-2	+	-
125	C	C3-1NC-4	+	-
126	C	C3-3NC-1	+	-
127	C	C3-4NC-1	+	-
128	E	E1-5NC-1	+	-
129	F	H1-2NC	+	-
130	F	H1-3NC-1	+	-
131	F	H1-3NC-2	-	-
132	F	H1-3NC-3	+	-
133	F	H1-3NC-4	+	-
134	F	H1-3NC-5	+	-
135	F	H1-4NC-1	+	-
136	F	H1-4NC-2	+	-
137	F	H1-5NC-2	+	-
138	F	H2-1NC-1	+	-
139	F	H2-1NC-2	+	-
140	F	H2-1NC-3	+	-
141	F	H2-2NC-1	+	-
142	F	H2-2NC-3	+	-

143	F	H2-5NC-1	+	-
144	F	H4-1NC-1	+	-
145	F	H4-1NC-3	+	-
146	F	H4-1NC-4	+	-
147	F	H4-1NC-5	+	-
148	F	H4-2NC-1	+	-
149	F	H4-2NC-2	+	-
150	F	H4-2NC-3	+	-
151	F	H4-2NC-4	+	-
152	F	H4-2NC-6	+	-
153	F	H4-3NC-2	+	-
154	F	H4-3NC-3	+	-
155	F	H4-3NC-4	+	-
156	F	H4-4NC-1	-	-
157	F	H4-4NC-2	+	-
158	F	H4-4NC-3	+	-
159	F	H4-4NC-4	+	-
160	F	H4-4NC-5	+	-
161	F	H4-5NC-1	+	-
162	F	H4-5NC-5	+	-
163	F	H5-1NC	+	-
164	F	H5-2NC-2	+	-
165	F	H5-3NC-2	+	-
166	F	H5-3NC-3	+	-
167	F	H5-5NC	+	-
168	G	I2-2NC-1	+	-
169	G	I2-4NC-1	+	-
170	G	I2-4NC-2	+	-
171	G	I2-5NC-1	+	-
172	G	I3-1NC-2	+	-
173	G	I3-3NC-1	+	-
174	G	I3-3NC-2	+	-
175	G	I3-4NC-1	+	-
176	G	I4-1NC-1	+	-
177	G	I5-4NC-2	+	-
178	G	I6-2NC-1	+	-
179	G	I6-3NC-1	+	-
180	G	I6-4NC-1	+	-
181	G	I6-5NC 2	+	-
182	G	I6-5NC-3	-	-
183	G	I6-5NC-4	+	-
184	G	I7-5NC-2	+	-
185	G	I7-5NC-3	+	-

186	H	J3-1NC-1	+	-
187	H	J3-3NC-2	+	-
188	H	J3-3NC-3	+	-
189	H	J3-4NC-2	+	-
190	H	J3-4NC-3	+	-
191	H	J5-1NC-1	+	-
192	H	J5-1NC-2	+	-
193	H	J5-2NC-1	+	-
194	H	J5-3NC-1	+	-
195	H	J5-3NC-2	+	-
196	H	J5-5NC-3	+	-
197	I	K1-1NC-1	+	-
198	I	K1-1NC-3	+	-
199	I	K1-1NC-5	+	-
200	I	K1-2NC-1	+	-
201	I	K1-2NC-2	+	-
202	I	K1-3NC-1	+	-
203	I	K1-4NC-1	+	-
204	I	K1-5NC-1	+	-
205	I	K2-2NC-2	+	-
206	I	K2-2NC-3	-	-
207	I	K2-4NC-1	+	-
208	I	K2-4NC-2	+	-
209	I	K2-4NC-3	+	-
210	I	K2-4NC-4	+	-
211	I	K2-5NC-2	+	-
212	I	K3-1NC-1	+	-
213	I	K3-2NC-1	+	-
214	I	K3-2NC-2	+	-
215	I	K3-3NC-1	+	-
216	I	K3-4NC-1	+	-
217	I	K3-4NC-2	+	-
218	I	K4-1NC-1	+	-
219	I	K4-1NC-2	+	-
220	I	K4-3NC-1	+	-
221	I	K4-4NC-1	+	-
222	I	K4-4NC-2	+	-
223	I	K4-5NC-1	+	-
224	I	K4-5NC-3	+	-
225	I	K5-1NC-1	+	-
226	I	K5-1NC-2	+	-
227	I	K5-2NC-1	+	-
228	I	K5-3NC-1	+	-

229	I	K5-3NC-2	+	-
230	I	K5-4NC-1	+	-
231	J	L1-2NC-1	+	-
232	J	L1-2NC-2	+	-
233	J	L1-2NC-5	+	-
234	J	L1-3NC-1	+	-
235	J	L1-3NC-4	+	-
236	J	L1-4NC-4	-	-
237	J	L2-1NC-1	-	-
238	J	L3-1NC-2	+	-
239	J	L3-1NC-4	+	-
240	J	L3-3NC-3	-	-
241	J	L3-4NC-1	+	-
242	J	L3-4NC-2	+	-
243	J	L3-4NC-3	+	-
244	J	L3-5NC-1	+	-
245	J	L3-5NC-2	+	-
246	J	L3-5NC-3	+	-
247	J	L3-5NC-4	+	-
248	J	L3-5NC-5	+	-
249	J	L4-1NC-1	+	-
250	J	L4-1NC-2	+	-
251	J	L4-1NC-3	+	-
252	J	L4-2NC-1	+	-
253	J	L4-3NC-2	+	-
254	J	L4-4NC-1	+	-
255	J	L4-4NC-3	+	-
256	J	L4-4NC-4	+	-
257	J	L4-4NC-5	+	-
258	J	L4-5NC-2	+	-
259	J	L4-5NC-5	+	-
260	J	L5-1NC-1	-	-
261	J	L5-1NC-2	+	-
262	J	L5-2NC-2	+	-
263	J	L5-3NC-1	+	-
264	J	L5-3NC-2	+	-
265	J	L5-3NC-3	+	-
266	J	L5-3NC-4	+	-
267	J	L5-3NC-5	+	-
268	J	L5-3NC-6	+	-
269	J	L5-4NC-2	+	-
270	J	L5-5NC-2	+	-
271	J	L5-5NC-3	+	-

272	K	M2-3NC-1	+	-
273	K	M2-4NC-1	-	-
274	K	M2-8NC-1	+	-
275	K	M3-6NC-1	-	-
276	K	M4-1NC-1	+	-
277	K	M4-2NC-1	+	-
278	K	M4-6NC-1	+	-
279	K	M4-8NC-1	-	-
280	K	M4-10NC-1	+	-
281	L	N1-5NC-1	+	-
282	L	N3-1NC-1	+	-
283	L	N3-2NC-2	+	-
284	L	N3-3NC-2	+	-
285	L	N3-4NC-1	+	-
286	L	N4-1NC-1	+	-
287	L	N4-1NC-2	+	-
288	L	N4-3NC-1	+	-
289	L	N4-4NC-2	+	-
290	L	N5-1NC-1	+	-
291	L	N5-5NC-1	+	-
292	L	N5-5NC-2	+	-
293	M	O1-4NC-2	+	-
294	M	O1-5NC-6	+	-
295	M	O2-2NC-2	-	-
296	M	O2-7NC-2	+	-
297	M	O3-1NC-1	+	-
298	M	O3-1NC-2	+	-
299	M	O3-1NC-3	+	-
300	M	O3-1NC-6	+	-
301	M	O3-3NC-1	+	-
302	M	O3-3NC-3	+	-
303	M	O3-4NC-1	+	-
304	M	O3-5NC-3	+	-
305	M	O3-6NC-1	+	-
306	M	O3-6NC-3	+	-
307	M	O3-7NC-2	+	-
308	M	O3-7NC-7	+	-
309	M	O3-8NC-1	+	-
310	M	03-9NC-1	+	-
311	M	O3-10NC-4	+	-

Supplementary table 4. PTR isolates used for host selective toxin production genotyping from four no-till wheat fields from Oklahoma during 2017.

Isolate Number	Field	Code	*Tox A*	*Tox B*
1	A	A2-1NC-1	+	-
2	A	A2-1NC-2	+	-
3	A	A2-2NC-2	+	-
4	A	A2-3NC-3	+	-
5	A	A2-3NC-4	+	-
6	A	A2-4NC-1	+	-
7	A	A3-2NC-2	+	-
8	A	A3-3NC-1	+	-
9	A	A4-1NC-1	+	-
10	A	A4-3NC-1	+	-
11	F	H1-1NC-1	+	-
12	F	H1-3NC-1	+	-
13	F	H1-3NC-3	+	-
14	F	H3-1NC-1	+	-
15	F	H3-4NC-3	+	-
16	F	H3-5NC-1	-	-
17	F	H3-5NC-2	+	-
18	F	H3-5NC-3	-	-
19	F	H4-4NC-2	+	-
20	F	H4-4NC-3	+	-
21	F	H5-3NC-1	+	-
22	F	H5-4NC-1	+	-
23	F	H5-4NC-2	+	-
24	I	K1-1NC-1	+	-
25	I	K1-1NC-3	+	-
26	I	K1-2NC-2	+	-
27	I	K1-2NC-3	+	-
28	I	K1-2NC-4	+	-
29	I	K1-2NC-5	+	-
30	I	K1-3NC-3	+	-
31	I	K1-4NC-1	+	-
32	I	K1-4NC-3	+	-
33	I	K1-4NC-4	+	-
34	I	K2-1NC-4	+	-
35	I	K2-2NC-3	+	-
36	I	K2-2NC-4	+	-
37	I	K2-3NC-2	+	-
38	I	K2-3NC-3	+	-
39	I	K2-3NC-4	+	-

40	I	K2-4NC-1	+	-
41	I	K2-4NC-2	+	-
42	I	K2-4NC-4	+	-
43	I	K3-1NC-3	+	-
44	I	K3-2NC-1	-	-
45	I	K3-3NC-2	+	-
46	I	K3-3NC-3	+	-
47	I	K3-4NC-1	+	-
48	I	K3-4NC-4	+	-
49	I	K4-1NC-1	+	-
50	I	K4-1NC-5	+	-
51	I	K4-2NC-4	+	-
52	I	K4-2NC-5	+	-
53	I	K4-3NC-1	+	-
54	I	K4-3NC-5	+	-
55	I	K4-4NC-1	+	-
56	J	L1-1NC-2	+	-
57	J	L1-3NC-2	+	-
58	J	L1-3NC-3	+	-
59	J	L1-5NC-2	+	-
60	J	L1-5NC-4	+	-
61	J	L2-1NC-1	+	-
62	J	L2-3NC-1	+	-
63	J	L2-3NC-3	+	-
64	J	L2-4NC-1	+	-
65	J	L3-2NC-2	+	-
66	J	L3-2NC-3	+	-
67	J	L3-3NC-2	+	-
68	J	L3-4NC-1	+	-
69	J	L3-5NC-2	+	-
70	J	L4-1NC-1	+	-
71	J	L4-1NC-3	+	-
72	J	L4-2NC-3	+	-
73	J	L4-2NC-5	+	-
74	J	L4-3NC-1	+	-
75	J	L4-4NC-2	+	-
76	J	L5-4NC-1	+	-
77	J	L5-5NC-1	+	-
78	J	L5-5NC-2	+	-

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